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## Part II

# Department of Labor

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Occupational Safety and Health  
Administration

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29 CFR Parts 1910, 1915 and 1926  
Occupational Exposure to Methylene  
Chloride; Final Rule

**DEPARTMENT OF LABOR****Occupational Safety and Health Administration****29 CFR Parts 1910, 1915 and 1926**

RIN 1218-AA98

**Occupational Exposure to Methylene Chloride**

**AGENCY:** Occupational Safety and Health Administration (OSHA), Department of Labor.

**ACTION:** Final rule.

**SUMMARY:** The Occupational Safety and Health Administration (OSHA) hereby amends its existing regulations for employee exposure to methylene chloride (MC), (also known as methylene dichloride, dichloromethane or DCM). OSHA has determined, based on animal and human data, that the current permissible exposure limits (PELs) allow employee exposure to a significant risk of material impairment of health. OSHA is reducing the existing 8-hour time-weighted average (TWA) exposure from 500 parts MC per million parts (ppm) of air to 25 ppm. Also, OSHA is deleting the existing ceiling limit concentration of 1,000 ppm and is reducing the existing short-term exposure limit from 2,000 ppm (measured over five minutes in any 2 hour period) to 125 ppm, measured as a 15-minute TWA. In addition, the Agency is setting an "action level" of 12.5 ppm, measured as an 8-hour TWA. The final rule also contains provisions for exposure control, personal protective equipment, employee exposure monitoring, training, medical surveillance, hazard communication, regulated areas, and recordkeeping. Together, these provisions will substantially reduce significant risk to the extent feasible. This standard applies to all employment in general industry, shipyards and construction. Small employers, for purposes of the Regulatory Flexibility Act, 5 U.S.C. 601, are defined as firms with fewer than twenty employees. The final standard will prevent an estimated 31 cancer deaths per year and an estimated three deaths per year from acute central nervous system and carboxyhemoglobinemic effects, and will also reduce cardiovascular disease and material impairment of the central nervous system. The estimated cost, on an annualized basis, is \$101 million per year.

**DATES:** This final rule becomes effective April 10, 1997.

**Compliance:** Start-up dates for specific provisions are set in

§ 1910.1052(n) of the regulatory text. However, affected parties do not have to comply with the information collection requirements in § 1910.1052(d) exposure monitoring, § 1910.1052(e) regulated areas, § 1910.1052(j) medical surveillance, § 1910.1052(l) employee information and training; and § 1910.1052(m) recordkeeping, until the Department of Labor publishes in the Federal Register the control numbers assigned by the Office of Management and Budget (OMB). Publication of the control numbers notifies the public that OMB has approved these information collection requirements under the Paperwork Reduction Act of 1995.

**Comments:** Interested parties may submit comments on the information collection requirements for this standard until March 11, 1997.

**ADDRESSES:** In compliance with 28 U.S.C. 2112(a), the Agency designates the Associate Solicitor for Occupational Safety and Health, Office of the Solicitor, Room S-4004, U.S. Department of Labor, 200 Constitution Avenue, NW., Washington, D.C. 20210, as the recipient of petitions for review of the standard.

Comments on the paperwork requirements of this final rule are to be submitted to the Docket Office, Docket No. ICR96-15, U.S. Department of Labor, Room N-2625, 200 Constitution Ave., NW., Washington D.C. 20210, telephone (202) 219-7894. Written comments limited to 10 pages or less in length may also be transmitted by facsimile to (202) 219-5046.

Copies of the referenced information collection request are available for inspection and copying in the Docket Office and will be mailed immediately to persons who request copies by telephoning Vivian Allen at (202) 219-8076. For electronic copies of the Methylene Chloride Final Standard and the Information Collection Request, contact OSHA's WebPage on Internet at <http://www.osha.gov/>.

**FOR FURTHER INFORMATION CONTACT:** Bonnie Friedman, Director, OSHA Office of Public Affairs, Room N-3647, U.S. Department of Labor, 200 Constitution Avenue, NW, Washington, D.C. 20210; Telephone (202) 219-8148.

**SUPPLEMENTARY INFORMATION:**

Collections of Information: Comment Request

The Department of Labor, as part of its continuing effort to reduce paperwork and respondent burden, conducts a preclearance consultation program to provide the general public and Federal agencies with an opportunity to comment on proposed and/or

continuing collections of information in accordance with the Paperwork Reduction Act of 1995 (PRA95) (44 U.S.C. 3506(c)(2)(A)). This program helps to ensure that requested data can be provided in the desired format, reporting burden (time and financial resources) is minimized, collection instruments are clearly understood, and the impact of collection requirements on respondents can be properly assessed. Currently, OSHA is soliciting comments concerning the proposed approval for the paperwork requirements of the Methylene Chloride Final Standard. Written comments should:

- Evaluate whether the proposed collection of information is necessary for the proper performance of the functions of the agency, including whether the information will have practical utility;
- Evaluate the accuracy of the agency's estimate of the burden of the proposed collection of information, including the validity of the methodology and assumptions used;
- Enhance the quality, utility, and clarity of the information to be collected; and
- Minimize the burden of the collection of information on those who are to respond, including through the use of appropriate automated, electronic, mechanical, or other technological collection techniques or other forms of information technology, e.g., permitting electronic submissions of responses.

**Background:** The Methylene Chloride Standard and its information collection requirements are designed to provide protection for employees from adverse health effects associated with occupational exposure to MC. The standard requires employers to monitor employee exposure to MC and inform employees of monitoring results. If monitoring results are above the 8-hour TWA PEL or the STEL, then employers must also inform employees of the corrective action that will be taken to reduce employee exposure to or below the 8-hour PEL or STEL. Employers may also be required to provide medical surveillance to employees who are or may be exposed to MC. Employers are also required to provide information and training to employees on the following: health effects of MC, specifics regarding use of MC in the workplace, the contents of the standard, and means the employee can take to protect themselves from overexposure to MC.

**Current Actions:** This notice requests public comment on the paperwork requirements in the Methylene Chloride Final Standard. The Agency previously sought clearance on three Methylene

Chloride Notice of Proposed Rulemaking Information Collection Requests: Shipyards, 1218-0177; Construction, 1218-0178; and General Industry, 1218-0179. Since the information requirements are identical for each industry, the Agency has combined these three packages into one entitled Methylene Chloride § 1910.1052, OMB number 1218-0179.

*Type of Review:* Revision of a currently approved collection.

*Agency:* Occupational Safety and Health Administration.

*Title:* Methylene Chloride § 1910.1052.

*OMB Number:* 1218-0179.

*Agency Number:* Methylene Chloride Docket Number H-71.

*Recordkeeping:* Employers must maintain employee medical records for at least the duration of employment plus thirty years. Employee exposure monitoring records must be maintained for at least 30 years. Objective data, data showing that any materials in the workplace containing MC will not release MC at levels which exceed the action level or the STEL under foreseeable condition of exposures, must be maintained as long as the employer is relying on the data in support of the initial monitoring exemption.

*Affected Public:* Business or other for-profit, Federal government, State and Local governments.

*Total Respondents:* 92,000.

*Frequency:* On Occasion.

*Total Responses:* Initial 719,948; Recurring 299,620.

*Average Time per Response:* 0.26 hour.

*Estimated Total Burden Hours:* Initial 188,728; Recurring 74,299.

*Estimated Total Burden Cost:* Initial \$32,496,380; Recurring \$12,282,420.

Comments submitted in response to this notice will be summarized and/or included in the request for the Office of Management and Budget approval of the information collection request; they will also become a matter of public record.

#### Federalism

This standard has been reviewed in accordance with Executive Order 12612, 52 FR 41685 (October 30, 1987), regarding Federalism. This Order requires that agencies, to the extent possible, refrain from limiting State policy options, consult with States prior to taking any actions that would restrict State policy options, and take such actions only when there is a clear constitutional authority and the presence of a problem of national scope. The Order provides for preemption of State law only if there is a clear

Congressional intent for the Agency to do so. Any such preemption is to be limited to the extent possible.

Section 18 of the Occupational Safety and Health Act (OSH Act), expresses Congress' clear intent to preempt State laws with respect to which Federal OSHA has promulgated occupational safety or health standards. Under the OSH Act, a State can avoid preemption only if it submits, and obtains Federal approval of, a plan for the development of such standards and their enforcement. Occupational safety and health standards developed by such State Plan-States must, among other things, be at least as effective in providing safe and healthful employment and places of employment as the Federal standards. Where such standards are applicable to products distributed or used in interstate commerce, they may not unduly burden commerce and must be justified by compelling local conditions (See section 18(c)(2)).

The final MC standard is drafted so that employees in every State will be protected by general, performance-oriented standards. States with occupational safety and health plans approved under section 18 of the OSH Act will be able to develop their own State standards to deal with any special problems which might be encountered in a particular state. Moreover, the performance nature of this standard, of and by itself, allows for flexibility by States and employers to provide as much leeway as possible using alternative means of compliance.

This final MC rule addresses a health problem related to occupational exposure to MC which is national in scope.

Those States which have elected to participate under section 18 of the OSH Act would not be preempted by this regulation and will be able to deal with special, local conditions within the framework provided by this performance-oriented standard while ensuring that their standards are at least as effective as the Federal Standard.

#### State Plans

The 23 States and two territories with their own OSHA-approved occupational safety and health plans must adopt a comparable standard within six months of the publication of this final standard for occupational exposure to methylene chloride or amend their existing standards if it is not "at least as effective" as the final Federal standard. The states and territories with occupational safety and health state plans are: Alaska, Arizona, California, Connecticut (for State and local

government employees only), Hawaii, Indiana, Iowa, Kentucky, Maryland, Michigan, Nevada, New Mexico, New York (for State and local government employees only), North Carolina, Oregon, Puerto Rico, South Carolina, Tennessee, Utah, Vermont, Virginia, the Virgin Islands, Washington, and Wyoming. Until such time as a State standard is promulgated, Federal OSHA will provide interim enforcement assistance, as appropriate, in these states and territories.

#### Unfunded Mandates

The MC final rule has been reviewed in accordance with the Unfunded Mandates Reform Act of 1995 (UMRA) (2 U.S.C. 1501 *et seq.*) and Executive Order 12875. As discussed below in the Summary of the Final Economic Analysis (FEA) (Section VIII of this document), OSHA estimates that compliance with the revised MC standard will require the expenditure of slightly more than \$100 million each year by employers in the private sector. Therefore, the MC final rule establishes a federal private sector mandate and is a significant regulatory action, within the meaning of Section 202 of UMRA (2 U.S.C. 1532). OSHA has included this statement to address the anticipated effects of the MC final rule pursuant to Section 202.

OSHA standards do not apply to state and local governments, except in states that have voluntarily elected to adopt an OSHA State Plan. Consequently, the MC standard does not meet the definition of a "Federal intergovernmental mandate" (Section 421(5) of UMRA (2 U.S.C. 658(5))). In addition, the Agency has concluded, based on review of the rulemaking record, that few, if any, of the affected employers are state, local and tribal governments. Further, OSHA has found that any impact on such entities would be insignificant. In sum, the MC standard does not impose unfunded mandates on state, local and tribal governments.

The anticipated benefits and costs of this final standard are addressed in the Summary of the FEA (Section VIII of this document), below, and in the FEA [Ex. 129]. In addition, pursuant to Section 205 of the UMRA (2 U.S.C. 1535), having considered a reasonable number of alternatives as outlined in this Preamble and in the FEA [Ex. 129], the Agency has concluded that the final rule is the most cost-effective alternative for implementation of OSHA's statutory objective of reducing significant risk to the extent feasible. This is discussed at length in the FEA [Ex. 129] and in the Summary and Explanation (Section X of

this document) for the various provisions of the MC standard.

## I. General

The preamble to the final rule on occupational exposure to Methylene Chloride (MC) discusses the events leading to the final rule, the physical and chemical properties of MC, the health effects of exposure, the degree and significance of the risk presented by MC exposure, the Final Economic Analysis and Regulatory Flexibility Analysis, and the rationale behind the specific provisions set forth in the final standard. The discussion follows this outline:

- I. General
- II. Pertinent Legal Authority
- III. Events Leading to the Final Standard
- IV. Chemical Identification
- V. Health Effects
- VI. Quantitative Risk Assessment
- VII. Significance of Risk
- VIII. Summary of the Final Economic Analysis
- IX. Environmental Impact
- X. Summary and Explanation of the Final Standard
  - A. Scope and Application
  - B. Definitions
  - C. Permissible Exposure Limits
  - D. Exposure Monitoring
  - E. Regulated Areas
  - F. Methods of Compliance
  - G. Respiratory Protection
  - H. Protective Clothing and Equipment
  - I. Hygiene Facilities
  - J. Medical Surveillance
  - K. Hazard Communication
  - L. Employee Information and Training
  - M. Recordkeeping
  - N. Dates
  - O. Appendices
- XI. Authority and Signature
- XII. Final Rule and Appendices
- Appendix A: Substance Safety Data Sheet and Technical Guidelines for Methylene Chloride
- Appendix B: Medical Surveillance for Methylene Chloride
- Appendix C: Questions and Answers—Methylene Chloride Control in Furniture Stripping

## II. Pertinent Legal Authority

The purpose of the Occupational Safety and Health Act, 29 U.S.C. 651 *et seq.* ("the Act") is to "assure so far as possible every working man and woman in the nation safe and healthful working conditions and to preserve our human resources." 29 U.S.C. § 651(b). To achieve this goal, Congress authorized the Secretary of Labor to promulgate and enforce occupational safety and health standards. U.S.C. §§ 655(a) (authorizing summary adoption of existing consensus and federal standards within two years of the Act's enactment), 655(b) (authorizing promulgation of standards pursuant to

notice and comment), 654(b) (requiring employers to comply with OSHA standards.)

A safety or health standard is a standard "which requires conditions, or the adoption or use of one or more practices, means, methods, operations, or processes, reasonably necessary or appropriate to provide safe or healthful employment or places of employment." 29 U.S.C. § 652(8).

A standard is reasonably necessary or appropriate within the meaning of Section 652(8) if it substantially reduces or eliminates significant risk, and is economically feasible, technologically feasible, cost effective, consistent with prior Agency action or supported by a reasoned justification for departing from prior Agency actions, supported by substantial evidence, and is better able to effectuate the Act's purposes than any national consensus standard it supersedes. See 58 FR 16612-16616 (March 30, 1993).

The Supreme Court has noted that a reasonable person would consider a fatality risk of 1/1000 to be a significant risk, and would consider a risk of one in one billion to be insignificant. *Industrial Union Department v. American Petroleum Institute*, 448 U.S. 607, 646 (1980) (the "Benzene decision"). So a risk of 1/1000 ( $10^{-3}$ ) represents the uppermost end of a million-fold range suggested by the Supreme Court, somewhere below which the boundary of acceptable versus unacceptable risk must fall. The Court further stated that "while the Agency must support its findings that a certain level of risk exists with substantial evidence, we recognize that its determination that a particular level of risk is significant will be based largely on policy considerations." See, e.g., *International Union, UAW v. Pendergrass*, 878 F.2d 389 (D.C. Cir. 1989) (formaldehyde standard); *Building and Constr. Trades Department, AFL-CIO v. Brock*, 838 F.2d 1258, 1265 (D.C. Cir. 1988) (asbestos standard).

A standard is technologically feasible if the protective measures it requires already exist, can be brought into existence with available technology, or can be created with technology that can reasonably be expected to be developed. *American Textile Mfrs. Institute v. OSHA* 452 U.S. 490, 513 (1981) ("ATMI"), *American Iron and Steel Institute v. OSHA*, 939 F.2d 975, 980 (D.C. Cir. 1991) ("AISI").

A standard is economically feasible if industry can absorb or pass on the cost of compliance without threatening its long term profitability or competitive structure. See *ATMI*, 452 U.S. at 530 n. 55; *AISI*, 939 F. 2d at 980.

A standard is cost effective if the protective measures it requires are the least costly of the available alternatives that achieve the same level of protection. *ATMI*, 453 U.S. at 514 n. 32; *International Union, UAW v. OSHA*, 37 F. 3d 665, 668 (D.C. Cir. 1994) ("LOTO III").

All standards must be highly protective. See 58 FR 16614-16615; *LOTO III*, 37 F. 3d at 668. However, health standards must also meet the "feasibility mandate" of Section 6(b)(5) of the Act, 29 U.S.C. 655(b)(5). Section 6(b)(5) requires OSHA to select "the most protective standard consistent with feasibility" that is needed to reduce significant risk when regulating health hazards. *ATMI*, 452 U.S. at 509.

Section 6(b)(5) also directs OSHA to base health standards on "the best available evidence," including research, demonstrations, and experiments. 29 U.S.C. § 655(b)(5). OSHA shall consider "in addition to the attainment of the highest degree of health and safety protection \* \* \* the latest scientific data \* \* \* feasibility and experience gained under this and other health and safety laws." *Id.*

Section 6(b)(7) of the Act authorizes OSHA to include among a standard's requirements labeling, monitoring, medical testing and other information gathering and transmittal provisions. 29 U.S.C. § 655(b)(7).

## III. Events Leading to the Final Standard

The present OSHA standard for MC requires employers to ensure that employee exposure does not exceed 500 ppm as an 8-hour TWA, 1000 ppm as a ceiling concentration, and 2000 ppm as a maximum peak for a period not to exceed five minutes in any two hours (29 CFR 1910.1000, Table Z-2). This standard was adopted by OSHA in 1971 pursuant to section 6(a) of the OSH Act, 29 U.S.C. 655, from an existing Walsh-Healey Federal Standard. The source of this Walsh-Healey Standard [Ex. 7-1] was the American National Standards Institute (ANSI) standard for acceptable concentrations of MC (ANSI-Z37.23-1969), which was intended to protect workers from injury to the neurological system including loss of awareness and functional deficits linked to anesthetic and irritating properties of MC which had been observed from excessive, acute or large chronic exposures to MC in humans and experimental animals.

In 1946, the American Conference of Governmental Industrial Hygienists (ACGIH) recommended a Threshold Limit Value (TLV) of 500 ppm for MC [Ex. 2]. In 1975, the ACGIH lowered the

recommended TLV to 100 ppm [Ex. 7-11].

In March 1976, the National Institute for Occupational Safety and Health (NIOSH) published "Criteria for a recommended standard for Methylene Chloride" [Ex. 2], which recommended a reduction of occupational exposures to MC to 75 ppm as an 8-hour TWA, and a lower peak exposure not to exceed 500 ppm. Further exposure reduction based on the ambient level of carbon monoxide was also recommended.

In February 1985, the National Toxicology Program (NTP) reported the final results of animal studies indicating that MC is a potential cancer causing agent [Ex. 7-8]. Subsequently, the U.S. Environmental Protection Agency (EPA), upon receipt of the NTP studies, initiated a risk assessment evaluation to determine whether or not MC presents an unreasonable risk to human health or the environment and to determine if regulatory actions are needed to eliminate or reduce exposures.

On May 14, 1985, EPA announced its determination that MC was a probable human carcinogen. EPA classified MC as Group B2, in accordance with its interim guidelines for cancer risk (49 FR 46294), and hence announced the initiation of a 180-day priority review (50 FR 20126) under section 4(f) of the Toxic Substances Control Act (TSCA). In meeting its mandate under section 4(f) of TSCA to initiate a regulatory action, on October 17, 1985, EPA published an Advance Notice of Proposed Rulemaking (ANPR) (50 FR 42037) for the purpose of collecting the necessary information required for initiating a rulemaking. In this notice, EPA established December 16, 1985, as its deadline for receiving comments.

On April 11, 1985, the U.S. Consumer Product Safety Commission (CPSC) released its risk assessment findings for MC and began to consider a regulatory action to ban MC containing products and to develop a voluntary hazard communication program for consumers.

On December 18, 1985, the U.S. Food and Drug Administration (FDA) published a proposal to ban the use of MC as an ingredient in aerosol cosmetic products (50 FR 51551). This proposal was based on a risk assessment that used the NTP animal data.

On July 19, 1985, Owen Bieber, President of International Union, United Automobile, Aerospace and Agricultural Implement Workers of America (UAW), petitioned OSHA to act expeditiously on reducing workers' exposure to MC. Specifically, Mr. Bieber requested that OSHA: (1) Publish a hazard alert; (2) issue an emergency temporary standard (ETS); and (3) begin work on a new

permanent standard for controlling MC exposure. Subsequently, the following unions joined UAW in petitioning OSHA to act on revising the current standard:

- A. International Union, Allied Industrial Workers of America;
- B. Glass, Pottery, Plastics and Allied Workers International Union;
- C. United Furniture Workers of America;
- D. The Newspaper Guild;
- E. Communication Workers of America; and
- F. United Steelworkers of America.

In March 1986, as a preliminary response to this petition, OSHA issued "Guidelines for Controlling Exposure to Methylene Chloride." That document, which was canceled by OSHA Notice ADM 8 (July 12, 1994), provided information to employers and workers on risks of MC exposure and methods for controlling such exposure [Ex. 8-11].

In April 1986, NIOSH published a Current Intelligence Bulletin #46 (CIB) on MC reflecting the findings of the NTP study [Ex. 8-26]. The CIB concluded that MC should be regarded as a potential occupational carcinogen and that exposure should be controlled to the lowest feasible level.

On August 20, 1986, the CPSC issued a proposed rule [51 FR 29778] "that would declare household products containing other than contaminant levels of MC to be hazardous substances." The CPSC noted the proposal was prompted by evidence that inhalation of MC vapor increased the incidence of various malignant and benign tumors in rats and mice. Accordingly, the Commission proposed to require that household products which can expose consumers to MC vapor be treated as hazardous substances and be labeled as provided by section 2(p)(1) of the Federal Hazardous Substances Act (FHSA) (15 U.S.C. 1261(p)(1)). The FHSA requires the use of labels which (1) indicate that exposure to a product may present a cancer risk; (2) explain the factors (such as level and duration of exposure) that control the degree of risk; and (3) explain the precautions to be taken.

On November 17, 1986, OSHA denied the petition for an Emergency Temporary Standard, but agreed that work on a permanent standard should commence [Ex. 3A]. On November 24, 1986, OSHA announced, in an Advance Notice of Proposed Rulemaking (ANPR) [51 FR 42257], that it was considering revision of the occupational health standard for MC. The Agency based this action on animal studies which indicated that the PEL of 500 ppm did

not provide adequate protection against potential cancer risks and other adverse health effects. The ANPR summarized OSHA's information regarding the production and use of MC, occupational exposure to MC, and the potential adverse health effects associated with MC exposure. In addition, the notice invited interested parties to submit comments, recommendations, data, and information on a variety of issues related to the regulation of MC. OSHA received 43 comments in response to the ANPR. Those comments are discussed, as appropriate, below.

On December 5, 1986, the FDA reopened the comment period for 30 days on the above-cited proposal to ban the use of MC in cosmetic products [51 FR 43935]. The reopening enabled interested parties to submit comments on studies received after the close of the initial comment period regarding MC comparative pharmacokinetics, metabolism, and genotoxicity.

On September 14, 1987, the CPSC issued a statement of interpretation and enforcement policy, in lieu of continuing with rulemaking, which expressed the Commission's determination that consumer products containing MC and capable of exposing consumers to significant amounts of MC may pose cancer risk to humans and, therefore, are subject to the above-described hazardous substance labeling requirements. The CPSC explicitly retained the option of resuming the rulemaking if voluntary compliance with and enforcement of the Commission's interpretation did not adequately induce firms to label their products appropriately.

In 1988, based on the response to the ANPR, OSHA began contacting small businesses and conducting a number of site visits, to develop a clear understanding of how revisions to OSHA's MC standard would affect small entities. For example, on April 27, 1989, OSHA participated in a NIOSH conference on MC controls for the furniture stripping industry (54 FR 11811, March 22, 1989) to learn how that industry, which is dominated by small businesses, was dealing with MC exposure. That conference focused on the progress of a NIOSH pilot program aimed at developing affordable engineering controls for the furniture stripping industry. OSHA continued to seek input from small businesses throughout the MC rulemaking, as discussed below in the Preamble and in the Final Economic Analysis [Ex. 129].

Also, in 1988, ACGIH officially lowered the TLV for MC to 50 ppm as an 8-hour TWA. OSHA considered whether the TLV recommended by the

ACGIH would be an appropriate OSHA standard. The ACGIH is a professional society devoted to administrative and technical aspects of occupational and environmental health. Voting members of ACGIH are scientists who work for government agencies or educational institutions. Every year the ACGIH adopts new or revised TLVs for several substances by a majority vote, not by consensus. OSHA has not adopted the MC TLV (50 ppm) as the 8-hour TWA PEL because the Agency's criteria for setting standards differ from those used by the ACGIH. OSHA standards must eliminate significant risks to the extent feasible, whereas the ACGIH sets limits under which it is believed that nearly all workers may be repeatedly exposed day after day without adverse health effects. Also, as evidenced by their "Documentation of the TLVs," the ACGIH does not perform quantitative risk assessments. This difference between OSHA and ACGIH practice is critical because the Supreme Court has required OSHA to perform quantitative risk assessments when data permit, and to use these assessments to set exposure limits.

On June 29, 1989, the FDA issued a final rule that banned the use of MC in cosmetic products [54 FR 27328]. The Agency based its final rule on scientific studies that showed inhalation of MC caused cancer in laboratory animals. The FDA concluded, accordingly, "that continued use of MC in cosmetic products may pose a significant risk to human health \* \* \*." The Agency considered comments and information regarding the application of a physiologically-based pharmacokinetic model to the prediction of human cancer risk. The FDA determined that the risk assessment developed using animal studies should not be changed to reflect the "pharmacokinetic and metabolic data and hypothesized GST metabolic mechanism of carcinogenicity."

On August 8, 1990, the Consumer Product Safety Commission (CPSC) issued a General Order (55 FR 32282) that required manufacturers, importers, packagers and private labelers of consumer products containing 1% or more of MC to report to the CPSC information on the labeling and marketing of those products. The CPSC indicated that the information obtained would aid the Commission in evaluating the CPSC's policy concerning the labeling of MC-containing products as hazardous substances, pursuant to the Federal Hazardous Substances Act.

On November 11, 1990, then-President Bush signed the Clean Air Act Amendments (CAAA) of 1990. Title VI

of the CAAA requires the phaseout of ozone-depleting chemicals by the year 2000 (section 604) and requires the EPA to determine which alternatives to ozone-depleting chemicals are safe for use (section 612). MC was among the potential substitutes studied by the EPA. In addition, section 112 of the CAAA requires the EPA to address the residual risks of MC and other specified Hazardous Air Pollutants (HAPs) by establishing Maximum Achievable Control Technology (MACT) standards. In particular, section 112(d) requires EPA to promulgate National Emission Standards for Hazardous Air Pollutants (NESHAP) (40 CFR part 63) over a 10-year period. In addition, EPA regulates MC as a priority pollutant under the Clean Water Act as amended (33 U.S.C. 1251, *et seq.*)

On February 12-13, 1991, EPA convened an international conference on "Reducing Risk in Paint Stripping" that was well attended by representatives of small businesses which use MC or its substitutes in a wide range of operations. OSHA actively participated in the workgroup and panel discussions to elicit information regarding the anticipated impacts of a revised MC standard on paint stripping operations.

OSHA determined, based on animal and human data, that the existing PELs for MC did not adequately protect employee health. Accordingly, on November 7, 1991, OSHA issued a notice of proposed rulemaking (NPRM) (56 FR 57036) to address the significant risks of MC-induced health effects. The proposed rule required employers to reduce occupational exposure to MC and to institute ancillary measures, such as employee training and medical surveillance, for further protection of MC-exposed workers. The provisions of the proposed rule are discussed in detail in the Summary and Explanation, Section X, below. The Agency published a correction notice on January 6, 1992 (57 FR 387). The NPRM solicited comments on the proposed rule and raised 48 specific issues to elicit information about MC health effects, use, and exposure controls, as well as input regarding the appropriateness and impacts of particular provisions. The written comment period, which ended on April 6, 1992, produced 58 comments, including several hearing requests.

On February 11, 1992, then-President Bush announced an accelerated phaseout schedule for ozone depleting substances and ordered the EPA to accelerate its review of substitutes (such as MC) whose use would reduce damage to the ozone layer.

On May 19, 1992, OSHA presented the MC proposal to the newly reconstituted Advisory Committee on Construction Safety and Health (ACCSH) for consultation. The Advisory Committee established a MC work group to generate information and recommendations regarding MC use and exposure in the construction industry.

In response to the hearing requests and to concerns raised by commenters, the Agency issued a notice of informal public hearing (57 FR 24438, June 9, 1992), which scheduled hearings to start in Washington, D.C. on September 16, 1992 and in San Francisco, California on October 14, 1992. That notice also reopened the written comment period until August 24, 1992. The hearing notice raised 16 issues, based on the NPRM comments, which solicited input regarding the human health risks of MC exposure and the impact of the proposed rule on MC users. San Francisco was selected as a hearing site to facilitate participation by small businesses, particularly foam blowers and furniture refinishers, for whom attendance at the Washington, D.C. hearing would have been economically burdensome.

On July 28, 1992, the MC work group's report was presented to the ACCSH and was adopted as the Advisory Committee's recommendation to OSHA. Based on the input from the ACCSH, OSHA issued a supplemental hearing notice (57 FR 36964, August 17, 1992) which raised MC use, exposure and control issues specific to the construction industry. The supplemental notice extended the deadline for submission of comments regarding the construction issues until September 22, 1992.

OSHA convened public hearings in Washington, D.C. on September 16-24, 1992 and in San Francisco on October 14-16, 1992, with Administrative Law Judge James Guill presiding. At the conclusion of the hearings, Judge Guill set a post hearing period for the submission of additional data, which ended on January 14, 1993, and for the submission of additional briefs, arguments and summations, which ended on March 15, 1993. The posthearing comment period elicited 35 comments.

On March 31, 1993, pursuant to section 112 of the CAAA, the EPA issued a notice (58 FR 16808) requesting information on the anticipated impacts of a National Emission Standard for Hazardous Air Pollutants (NESHAP) for the halogenated solvent cleaning-vapor degreasing source category. This notice characterized MC as the third most commonly used halogenated solvent,

based on 1991 data. On November 29, 1993, the EPA issued a notice of proposed rulemaking (58 FR 62566) describing MACT rules for the use of MC and other HAPs in halogenated solvent cleaning-vapor degreasing operations.

On March 11, 1994, OSHA reopened the rulemaking record for 45 days (59 FR 11567) to receive public comment on reports related to engineering controls for MC exposure in the furniture refinishing industry, MC carcinogenicity, and the availability of water-based substitutes for MC-based adhesives in the manufacture of flexible foam products. In particular, OSHA solicited input regarding the extent to which it was feasible for small businesses with furniture stripping operations to comply with the proposed PELs using engineering controls addressed in an OSHA contractor's report [Ex. 114]. The limited reopening, which ended on April 25, 1994, elicited 29 comments.

OSHA has evaluated the impact of the final rule on the identified application groups (except for farm equipment [Ex. 115-23], insofar as this rulemaking does not address agricultural employment). The Agency's analysis and conclusions are presented in the Final Economic Assessment for this rulemaking [Ex. 129], summarized in Section VIII, below.

On March 18, 1994, the EPA issued a final rule (59 FR 13044) which addressed the use of MC as a substitute for ozone-depleting chemicals being phased out under section 612 of the CAAA of 1990. The EPA has found the use of MC to be acceptable in the production of flexible polyurethane foam; polyurethane integral skin foams; metal cleaning; electronics cleaning; precision cleaning; and adhesives, coatings and inks. That Agency expressed concern regarding MC toxicity, stating "methylene chloride use will be subject to future controls for hazardous air pollutants under Title III section 112 of the CAA. In addition, use of the compound must conform to all relevant workplace safety standards \* \* \* Use is also subject to waste disposal requirements under RCRA (59 FR at 13088)." The EPA also noted that it is encouraging companies to decrease emissions of MC through the "30/50" pollution prevention program, under which companies voluntarily commit to reduce emissions 33 percent by the end of 1992 and 50 percent by the end of 1995 (59 FR at 13093).

On April 21, 1994, the Department of Housing and Urban Development (HUD) issued a notice (59 FR 19084) announcing that funds were available

for the removal of lead-based paint. That notice explicitly provided that paint removal activities funded by HUD could not use products containing MC.

On May 31, 1994, Judge Guill closed and certified the hearing record for OSHA's MC rulemaking.

Pursuant to section 112(d) of the CAAA, the EPA has already finalized NESHAP rulemakings that cover halogenated solvent cleaning (59 FR 61801, December 4, 1994, 40 CFR part 63, subpart T), aerospace manufacture and rework facilities (September 1, 1995, 40 CFR part 63, subpart ) and wood furniture manufacturing (60 FR 62930, December 7, 1995, 40 CFR part 63, subpart JJ). MC-related NESHAP proceedings for several industries (e.g., pharmaceuticals, flexible polyurethane foam, polycarbonates and nylon 6 are currently underway.

Pursuant to its CAAA, CWA, RCRA and PPA mandates, EPA has proposed effluent limitation guidelines for the pharmaceutical industry (60 FR 21592, May 2, 1995) which characterize MC as one of the most significant priority pollutants to be addressed under the CWA. In particular, EPA has addressed the use of stream stripping and distillation technology to recover MC from wastewater for reuse or sale for use in other industries. That Agency has also proposed requirements for compliance monitoring of MC that, due to dilution with wastewater, would be found at levels below current analytical limits of detection.

OSHA has attempted to consider the foreseeable impact of EPA action on the use of MC because EPA-driven changes in such use would affect the data on which OSHA relies to estimate the impact of this final rule. In brief, while EPA action to reduce HAP exposure may encourage employers to reduce or eliminate MC use, simultaneous EPA efforts to reduce the emission of ozone-depleting chemicals may encourage employers to maintain or increase MC use. Given the time frame for EPA action and that Agency's need to coordinate proceedings that arise from several statutory mandates, it is inappropriate to draw conclusions regarding the impact of EPA regulatory action on the need for OSHA action.

OSHA has also consulted with EPA to determine whether any potential overlapping or conflicting requirements exist in OSHA's MC standard and various EPA NESHAPs, and has committed to continue working with EPA on future NESHAP compliance issues. OSHA discussed the MC regulation with project officers for all recent, current and planned NESHAPs projects and has determined that there

are no overlapping or conflicting requirements in the NESHAPs and OSHA's MC standard. Indeed, employers can choose among a variety of means to comply which would not entail any conflict in OSHA and EPA regulations.

In particular, OSHA conducted a thorough analysis of the EPA Solvent Degreasing NESHAP. OSHA determined, and EPA agreed, that there are no conflicting requirements in the two regulations. OSHA does not require or recommend specific compliance strategies. One common method of reducing worker exposure is local exhaust ventilation. In addition, some of the alternative compliance strategies suggested in the EPA solvent degreasing NESHAP include reducing room draft. OSHA has determined that even if an employer chooses reducing room draft as its compliance strategy for the EPA NESHAP, employers may use some local exhaust ventilation to reduce worker MC exposures and still be in compliance with both the OSHA MC standard and the EPA NESHAP. There are also other combinations of compliance strategies that can be utilized to comply with both regulations. OSHA plans further discussion of this issue in its compliance assistance documents. The purpose of these documents is to assist employers in selecting among the many appropriate control strategies which satisfy requirements under both OSHA and EPA regulations.

On October 25, 1995, OSHA reopened the rulemaking record (60 FR 54462) to obtain input regarding studies submitted by the Halogenated Solvents Industry Alliance (HSIA) [Ex. 118-125] which address the use of animal data to estimate human cancer risk from MC exposure. The comments received on those studies [Exs. 126-1 through 126-37] are discussed in relation to the Quantitative Risk Assessment (Section VI), below.

The rulemaking record contains 129 exhibits, and 2717 pages of hearing transcript. A wide range of employees, employers, union representatives, trade associations, government agencies and other interested parties contributed to the development of the rulemaking record. The Agency appreciates these efforts to help OSHA develop a record that provides a sound basis for the promulgation of this final rule.

Throughout the ten years since OSHA initiated MC proceedings, the Agency has sought and evaluated input regarding the anticipated impact of a MC health standard on small entities. For example, Issue K of OSHA's Advance Notice of Proposed

Rulemaking for MC (ANPRM) (51 FR 42257, November 24, 1986) solicited comments, recommendations, data and information regarding the anticipated impacts of a MC standard on small entities. Responses from manufacturers of flexible polyurethane foam [Exs. 10-4 and 10-17] and industrial paint removers [Ex. 10-7] indicated that rulemaking regarding MC would affect small entities. Based on the response to the ANPRM, OSHA initiated contacts with small businesses and conducted a number of site visits, to develop a clear understanding of how revisions to OSHA's MC standard would affect small entities.

Based on OSHA's contacts with small business and the response to the ANPRM, the Preliminary Regulatory Impact Analysis (PRIA) for the MC NPRM (56 FR 57036, November 7, 1991) considered small firms to be those with fewer than 20 total employees. In addition, the PRIA estimated that 45 percent of establishments using MC were "small businesses."

Issue 25 of the NPRM for MC stated that OSHA had analyzed the impacts of the proposed rule on small businesses and had adapted the standard to take into account the circumstances of small businesses, where appropriate. The performance-oriented language covering the demarcation of regulated areas (proposed paragraph (e)(4)) and the 30/10 days of exposure thresholds for medical surveillance (proposed paragraph (i)(1)(ii)) reflected the Agency's determination to avoid imposing unnecessary burdens on small entities. In addition, Issue 25 solicited information regarding anticipated small business impacts so that OSHA could update the initial regulatory flexibility analysis performed pursuant to 5 U.S.C. 604 of the Regulatory Flexibility Act.

Small businesses, particularly in the furniture refinishing [Exs. 19-1, 19-4, 19-6, 19-8, 19-10 and 19-11] and polyurethane foam blowing industries [Ex. 19-3], expressed concern that the proposed rule would impose excessive compliance burdens on their operations. Based in part on these concerns, the Agency convened informal public hearings (57 FR 24438, June 9, 1992) in Washington, D.C. and San Francisco, CA. San Francisco was selected as a hearing site to facilitate participation by small businesses, particularly foam blowers and furniture refinishers, for whom attendance at the Washington, D.C. hearing would have been economically burdensome.

Hearing Notice Issue 8 solicited comments and testimony, with supporting documentation, regarding the impact of the proposed rule on small

businesses, particularly in the furniture refinishing sector. A significant number of small businesses participated in the Washington, D.C. and San Francisco hearings, providing OSHA with useful testimony and posthearing submissions. For example, Harold Markey of the Markey Restoration Company proposed [Tr. 2660, 2672, 10/16/92] that "furniture refinishing businesses be exempt from [25 ppm PEL] due to the financial hardship that enforcement would cause." In addition, Mr. Markey expressed appreciation for OSHA's efforts to facilitate his participation in the hearing. As discussed above, OSHA subsequently solicited (59 FR 11567, March 11, 1994) additional input regarding the extent to which it was feasible for small businesses with furniture stripping operations to comply with the proposed PELs using the engineering controls addressed in an OSHA contractor's report [Ex. 114].

OSHA has had numerous contacts with furniture refinishers, particularly with members of the National Association of Furniture Refinishers and Refurbishers (NAFRR), the trade association for the industry. In 1994, OSHA was represented at the NAFRR's annual conference in Williamsburg, VA. The Agency has continued to provide assistance to NAFRR members and other furniture refinishers regarding appropriate industrial hygiene measures for workplaces where MC is used. For example, OSHA has disseminated information about the engineering controls developed by NIOSH for the furniture stripping industry. OSHA will continue to strive for a cooperative relationship with the small businesses affected by the MC final rule through careful compliance with the Small Business Regulatory Enforcement Fairness Act (SBREFA) (5 U.S.C. Chapter 8) and the Regulatory Flexibility Act (5 U.S.C. 601, *et seq.*), as amended. In addition, the Agency's "Outreach Program" for the MC final rule will involve a commitment of significant consultation and other resources by OSHA and other concerned parties, building on the relationships established during the rulemaking.

OSHA has developed a multifaceted outreach plan to provide information and compliance assistance to the regulated community. In particular, OSHA:

- Has developed a booklet which summarizes the provisions of the MC standard;
- Has developed a compliance directive for the MC standard which answers compliance-related questions about the MC standard;

- Is developing compliance guides directed at assisting small businesses in complying with the MC standard, consistent with section 212 of the Small Business Regulatory Enforcement Fairness Act of 1996;
- Has recruited interested trade associations to assist in the distribution of MC standard-related information, and the convening of workshops to help small businesses understand available compliance strategies;
- Has spoken to trade association meetings and distributed MC standard-related materials;
- Has contacted manufacturers of MC to develop a strategy for inclusion of OSHA MC-standard information in existing product stewardship programs; and
- Is working with individuals interested in conducting workshops for impacted industries, such as polyurethane foam manufacturers and furniture refinishers, to train small businesses on compliance with OSHA and EPA regulations.

All 50 states and the territories covered by the OSH Act provide free consultation services for small businesses to assist them in achieving compliance with OSHA standards. Those services are funded by federal OSHA but supplied by the states in state plan states and by private contractors in other areas. Those consultation services will provide free assistance for small business so it will be easier to come into compliance with the MC standard.

OSHA will also set up Cooperative Assessment Programs (CAP's) for individual employers to assist them in achieving compliance in a reasonable manner. In a CAP, an OSHA industrial hygienist works with the employer and employee representatives, to determine a reasonable number of cost-effective engineering controls and work practices to bring the employer into compliance. A reasonable schedule is determined for the implementation of those controls. Good faith efforts to implement a CAP are generally considered to be in compliance with the provisions of the standard. OSHA has had success in implementing CAP's for the arsenic, lead and other standards. Employers have found that working with OSHA or CAP's has led to cost effective compliance with OSHA standards.

#### IV. Chemical Identification

Methylene chloride (MC), also called dichloromethane (DCM) [Chemical Abstracts Service Registry Number 75-09-2] is a halogenated aliphatic hydrocarbon with a chemical formula of  $\text{CH}_2\text{Cl}_2$ , a molecular weight of 84.9, a



boiling point of 39.8°C (104°F) at 760 mm Hg, a specific gravity of 1.3, a vapor density of 2.9 and a vapor pressure of 350 mm Hg at 20°C (68°F).

Concentration of MC in saturated air at 25°C reaches 550,000 ppm. MC has low water solubility (1.3 gm per 100 gm of water at 20°C), an extensive oil and fat solubility, and a low flammability potential. It is used as a flame suppressant in solvent mixtures (lower explosive limit of 12% and upper explosive limit of 19%). It is a colorless volatile liquid with a chloroform-like odor and its odor threshold varies between 100 and 300 ppm. Contact with strong oxidizers, caustics and active metal powder may cause explosions and fires. Decomposition products during combustion or fire include phosgene, hydrogen chloride and carbon monoxide.

## V. Health Effects

### A. Introduction

The toxicology of MC is summarized below. A more detailed review of MC toxicology can be found in the NPRM [56 FR 57036].

### B. Absorption and Disposition of Methylene Chloride

Inhalation is the most significant route of entry for MC in occupational settings. The quantity of MC taken into the body depends on the concentration of MC in inspired air, the breathing rate, the duration of exposure to MC, and the solubility of MC in blood and tissues. Because MC is volatile, inhalation exposures to MC can be quite high, especially in poorly ventilated spaces.

Dermal absorption of MC is a slow process relative to inhalation. In the NPRM, OSHA described the rate of skin

absorption of pure MC as insignificant relative to inhalation. In contrast, Mr. Harvey Clewell, in comments prepared for the U.S. Navy [Ex. 19–59], stated that substantial occupational exposure could occur through the dermal route when the employee is exposed to high concentrations of MC vapor and protective clothing is not worn [Ex. 19–59]. Mr. Clewell provided a physiologically-based pharmacokinetic (PBPK) model to describe the potential absorption through skin exposed to high vapor concentrations of MC. Where the employee is protected from inhalation exposure by use of an air-supplied respirator and the skin (exposed surface area = two hands) is unprotected in high MC-vapor concentrations, the primary route of exposure in this case will be dermal exposure. Mr. Clewell has determined that sufficient MC may be absorbed by the dermal route over an 8-hour shift to give an internal concentration which would exceed that experienced by workers exposed to MC through inhalation of 25 ppm for 8 hours.

In the NPRM, OSHA also indicated that the burning sensation associated with dermal exposure to liquid MC would likely lead employers and employees to limit skin absorption. However, exposure to high concentrations of vapor may not be associated with a burning sensation, and there is evidence in the record [Tr. 2468–70, 10/15/92] to suggest that employees are exposed to liquid MC without protective clothing. OSHA believes that dermal exposure to liquid and high vapor concentrations of MC should be limited to the extent feasible to protect the employee from overexposure. For this reason, in this

standard OSHA has required that employers provide personal protective clothing and equipment appropriate to the hazard. For example, if an employee will be at risk of hand contact with liquid MC, impermeable gloves must be provided.

### C. Metabolism of MC

Once MC is absorbed into the body, it is widely distributed in the body fluids and in various tissues. The uptake and elimination of MC has been well described in human and animal studies [Exs. 7–156, 7–157, 7–174].

The carcinogenic mechanism of action for MC has not been clearly established. Although it has not been proven whether MC is carcinogenic through a genotoxic or non-genotoxic mechanism, current evidence supports the hypothesis that MC is a genotoxic carcinogen. Genotoxic carcinogens typically are reactive compounds or metabolized to reactive compounds. MC is unreactive in the body until it is metabolized. Therefore, many investigators believe that one or more of the metabolites of MC, and not MC itself, is the ultimate carcinogen.

It has been established by Kubic and Anders [Ex. 7–167] and Ahmed and Anders [Ex. 7–25] that MC is metabolized by rat liver enzymes *in vitro* by two distinct pathways. The first pathway is the mixed function oxidase system (MFO pathway) associated with the microsomal cell fraction and the second is the glutathione dependent pathway localized primarily in the cytoplasm and mediated by glutathione-S-transferase (GST pathway). The metabolism of MC is illustrated in Figure 1.

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## Metabolism of Methylene Chloride

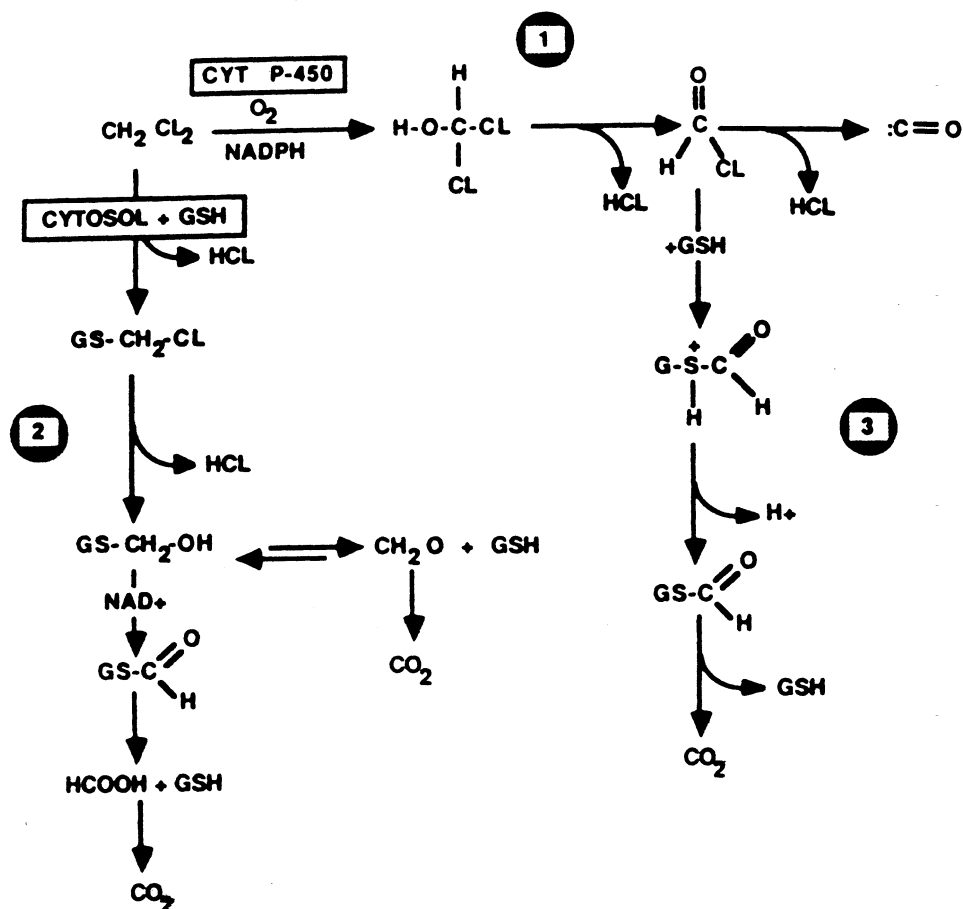


Figure V-1. Proposed metabolic pathways for methylene chloride metabolism. (Adapted from Andersen et al. (1987) [Ex. 7-125])

The MFO pathway metabolizes MC via a cytochrome-P450 dependent oxidative dehalogenation [Ex. 7-167] which produces formyl chloride. The formyl chloride decomposes to give chloride ion and carbon monoxide. It has been postulated that if the MFO pathway contributes to the carcinogenicity of MC, it is through the production of the reactive compound, formyl chloride. The end product of the MFO pathway, carbon monoxide, can be detected in the blood and breath of humans and animals exposed to MC, and has been used as a surrogate measure of MC exposure in humans.

The GST pathway metabolizes MC to formaldehyde and chloride ions via a postulated S-chloromethylglutathione conjugate [Ex. 7-25]. Formaldehyde is further metabolized to carbon dioxide in mammalian systems. Potential reactive metabolites in this pathway are the S-chloromethylglutathione conjugate and formaldehyde (known to react with protein, RNA and DNA).

Animal data indicate that the MFO pathway is saturated at ambient concentrations less than 500 ppm, while the GST pathway remains linear throughout the exposure levels examined [Exs. 7-161, 7-171]. Saturation of the MFO pathway in humans has been estimated to occur at a level which is within the range of the animal data (estimates range from 200 to 1000 ppm MC) [Exs. 7-114, 7-115, 8-32]. The GST pathway is not thought to be saturated for any of the species investigated at doses up to 4000 ppm.

#### D. Carcinogenicity

The evidence for the carcinogenicity of MC has been derived from mutagenicity studies, animal bioassays and human epidemiological studies. OSHA analyzed data from each of these sources in determining that MC is carcinogenic to test animals and a potential occupational carcinogen. The evidence that OSHA evaluated in making this determination is summarized below. Additional evidence pertaining to the hazard identification of MC is discussed in the Quantitative Risk Assessment, Section VI, below.

##### 1. Mutagenicity Studies

Mutagenicity and genotoxicity studies are useful in describing the possible carcinogenic mechanism of action of MC. Evidence for the interaction of MC or MC metabolites with DNA (producing mutations or toxicity) is consistent with a genotoxic mechanism for the carcinogenic action of MC, rather than a non-genotoxic action (i.e., by acting as a promoter, increasing cell turnover). The EPA reviewed the literature on the

mutagenic potential of MC in their "Health Assessment Document for Dichloromethane (Methylene Chloride)" (HAD) [Ex. 4-5] and studies conducted by ECETOC in the "Technical Analysis of New Methods and Data Regarding Dichloromethane Hazard Assessments" [Ex. 7-129].

As described in the MC Notice of Proposed Rulemaking (56 FR 57036), the documentation of positive responses in the production of mutations in bacteria, yeast and *Drosophila*, chromosomal aberrations in CHO cells and sister chromatid exchanges (SCE) in CHO and V79 cells and equivocal responses in other systems indicated the potential genotoxicity of MC.

A paper submitted to the record by Dr. Trevor Green [Ex. L-107], for the Halogenated Solvents Industry Alliance (HSIA), investigated the role of metabolites of the GST pathway in the bacterial mutagenicity of MC. The authors of this study found that in glutathione-deficient strains of *Salmonella typhimurium* there was approximately a two-fold decrease in mutations. Mutation rates returned to normal when bacteria were supplemented with exogenous glutathione. They also investigated whether individual metabolites in the GST pathway were likely to be responsible for mutagenesis. Experiments in *S. typhimurium* strains were consistent with the S-chloromethylglutathione conjugate as the mutagenic moiety. Experiments in *Escherichia coli* strains implicated formaldehyde as the active mutagen. Overall, these results support the hypothesis that MC may act as a genotoxic carcinogen, but the ultimate reactive species still remains to be identified.

Dillon et al. [Ex. 21-89] also conducted experiments on the mechanism of MC mutagenicity in bacterial cells, using wild type and glutathione-deficient *Salmonella typhimurium* TA100. Dose-related increases in mutagenicity were observed with and without metabolic (cytosolic or microsomal) activation. The authors characterized the mutagenicity as marginally highest in the presence of cytosol at the highest MC concentrations. The glutathione-deficient strain was slightly less responsive to MC-induced mutation than the wild type. In contrast to the study by Green, Dillon et al. found that MC mutagenicity was not appreciably enhanced by the addition of microsomal or cytosolic liver fractions or exogenous glutathione. They concluded that it was not clear to what extent, if any, glutathione was involved in MC

mutagenicity, and noted that " \* \* \* the residual glutathione present in the glutathione-deficient strain may have been sufficient to facilitate the mutagenic responses observed."

The differing results in these studies suggest that the exact mechanism of MC mutagenicity, even in bacterial cells, has not been determined with certainty. However, OSHA has concluded that the evidence that MC is genotoxic is compelling. Additional studies supporting classification of MC as a genotoxin were submitted to the Agency in late 1995 and are discussed in the Quantitative Risk Assessment, Section VI, below.

##### 2. Animal Studies

The evidence for the carcinogenicity of MC has been derived primarily from data obtained in chronic toxicity studies in rodents. Table V-1 contains a summary of the major bioassays. These bioassays have been conducted in three rodent species (rat, mouse and hamster) using two routes of administration (oral and inhalation) and a wide range of doses (from 5 mg/kg/d, oral to 4000 ppm inhaled for 6 hr/d, 5 d/wk).

The National Toxicology Program conducted two 2-year inhalation bioassays [Ex. 7-8] using B6C3F1 mice and Fischer 344 rats. In the NTP mouse study [Ex. 7-8], groups of 50 male and 50 female B6C3F1 mice were exposed to 0, 2000 or 4000 ppm MC, 6 hr/day, 5 d/wk for 102 weeks. All animals were necropsied and examined histopathologically.

Treated male and female mice had increased incidences of alveolar or bronchiolar adenomas and carcinomas as compared with control animals. In addition, there was an increased number of lung tumors per tumor-bearing animal (multiplicity of tumors) with increasing dose of MC.

In the liver, the toxic effects of MC were expressed as cytologic degeneration in male and female mice which was not present in the controls. An increased incidence of hepatocellular adenomas and carcinomas (combined) was observed in male mice. The incidence of hepatocellular carcinomas in male mice was statistically significantly increased at 4000 ppm. Female mice also experienced dose-related increases in the incidences of hepatocellular adenomas and carcinomas. An increased multiplicity of liver tumors was also found in both male and female mice.

TABLE V-1.—METHYLENE CHLORIDE LIFETIME BIOASSAYS

| Reference              | Species/strain                 | Route and dosing schedule         | Dosage (No. of animals)   | Comments  |
|------------------------|--------------------------------|-----------------------------------|---|---|
| NTP (1985) .....       | B6C3F <sub>1</sub> mouse ..... | Inhalation 6 hr/day, 5 days/week. | 0, 2000, 4000 ppm (50 mice/ sex/ dose).   | Lung and liver tumors both sexes, both doses.   |
| Serota (NCA) (1986).   | B6C3F <sub>1</sub> mouse ..... | Daily in water .....              | 0 (125M, 100F), 60 (200M, 100F), 125 (100M, 50F), 185 (100M, 50F), and 250 (125M, 50F) mg/kg/d. | No tumors observed.   |
| NTP (1985) .....       | Fischer 344 rat .....          | Inhalation 6 hr/day, 5 days/week. | 0, 1000, 2000 and 4000 ppm (50 rats/sex/dose).  | Mammary and integumentary fibromas and fibrosarcomas in both sexes.                   |
| Burek (DOW) (1980).    | Sprague-Dawley rat .....       | Inhalation 6 hr/day, 5 days/week. | 0, 500, 1500 and 3500 ppm (95 rats/sex/dose).   | Malignant salivary gland tumors at 3500 ppm, dose-related increase in mammary tumors. |
| Nitschke (DOW) (1982). | Sprague-Dawley rat .....       | Inhalation 6 hr/day, 5 days/week. | 0, 50, 200 and 500 ppm (70 rats/ sex/dose).   | No tumors observed.   |
| Serota (NCA) (1986).   | Fischer 344 rat .....          | Daily in water .....              | 0, 5, 50, 125 and 250 mg/kg/d (135/sex at 0, 85/sex/dose).                                      | No tumors observed.   |
| Burek (DOW) (1980).    | Syrian Golden hamster ..       | Inhalation 6 hr/day, 5 days/week. | 0, 500, 1500, 3500 ppm (90 hamsters/sex/ dose).   | No tumors observed.   |

The dose-related increase in the incidence of lung and liver tumors in mice, and the increased multiplicity of these tumors, present the strongest evidence for the carcinogenicity of MC. NTP concluded that, based on the evidence from these lung and liver tumors, there was clear evidence of the carcinogenicity of MC in both male and female mice.

In a second two-year bioassay, the NTP examined the effects of inhalation of MC at 0, 1000, 2000 and 4000 ppm in F344 rats [Ex. 7–8]. Body weights of all exposure groups were comparable. The highest dose female rats experienced reduced survival after 100 weeks of exposure.

The incidence of mammary tumors in the high dose group in both sexes was statistically significantly higher than in control animals (concurrent and historical). The incidence of mammary fibroadenomas alone and the combined incidence of fibroadenomas and adenomas in male and female rats occurred with statistically significant positive trends. When subcutaneous fibromas or sarcomas in the male rat, which were believed to have originated in the mammary chain, were included in comparisons, differences between control and exposed animals were even greater.

MC-exposed male and female rats also showed increased incidence of liver effects, characterized by hemosiderosis, hepatocytomegaly, cytoplasmic vacuolization and necrosis. Neoplastic nodules alone and combined incidence of neoplastic nodules and hepatocellular carcinomas in female rats occurred with significant positive trends by the life table test. Pair-wise comparisons did not indicate

statistically significant effects at any one dose. Although this is suggestive of a carcinogenic response in the female rat liver, NTP did not use this response in their determination of the carcinogenicity of MC.

NTP based its determination of the carcinogenicity of MC in the rat on the mammary tumor incidence data. NTP has concluded that the increased incidences of mammary gland tumors in the female rats provided clear evidence of carcinogenicity and, in the male rats, some evidence of carcinogenicity.

The Dow Chemical Company [Ex. 7–151] conducted experiments in which Sprague-Dawley rats and Syrian Golden hamsters were exposed to 0, 50, 1500 or 3500 ppm MC, 6 hr/d, 5 d/wk for 2 years. A dose-related statistically-significant increase in the number of mammary tumors per tumor-bearing female rat was observed. These results support the NTP findings of increased mammary tumors in F344 rats. The background mammary tumor response in the Sprague-Dawley rat is higher than in F344 rats, so a quantitative analysis of risk is easier to perform on the data from the NTP study.

A statistically significant increase in male rat salivary tumors was also observed in this study, although the authors believed that this response should be discounted because of the presence of sialodacryoadenitis virus in the rats. OSHA believes that the presence of this virus in the rats would complicate the interpretation of the data, and so has relied on the NTP studies for its quantitative risk assessments.

No statistically significant excess incidence of tumors was observed in either sex of hamsters at any exposure

level. This suggests that hamsters are less sensitive to the carcinogenic effects of MC than either mice or rats. Metabolism data gathered in hamsters indicate that hamsters have less capability to metabolize MC by the GST pathway than rats or hamsters (or humans). This correlation between lack of GST metabolism capacity and lack of tumor response supports the hypothesis that GST metabolism is important in MC carcinogenesis and also indicates that it would not be protective to use the hamster response to MC as the basis for a carcinogenic risk assessment.

A second inhalation study in Sprague-Dawley rats conducted by investigators at Dow Chemical [Ex. 7–173], with exposures up to 500 ppm, showed an increase in the number of mammary tumors per tumor-bearing animal in female rats at the highest dose level only. This study extended the finding of excess mammary tumors in rats to the 500 ppm level. However, because of the high background rates of mammary tumors in Sprague-Dawley rats, the NTP study showed a clearer dose-response relationship between MC exposure and incidence of mammary tumors.

In a study conducted for the National Coffee Association [Ex. 7–180], no statistically significant increased incidence of tumors was observed in B6C3F<sub>1</sub> mice or F344 rats exposed to up to 250 mg/kg/d MC in drinking water. These studies used the drinking water route of exposure instead of inhalation and exposed animals to lower doses (on an mg/kg/d basis) than the NTP and high-dose Dow studies. These factors most likely accounted for the lack of a positive tumor response. The NCA studies were used by Reitz et al. in the development of the physiologically-

based pharmacokinetic models for MC. Specifically, these studies helped to determine that the lack of tumor development was consistent with model predictions of the amount of GST metabolites in lung and liver of mice and that the MFO pathway was most likely not primarily responsible for the mouse tumor response.

The Agency believes that the NTP studies show the clearest evidence of a carcinogenic effect of MC and has used these studies as the basis of its risk assessment for the following reasons: (1) The studies were well conducted and underwent extensive peer review. (2) The inhalation route of exposure was used, which is the most appropriate route for extrapolation to occupational exposures. (3) Dose-related, statistically significant increases in tumor incidence were observed in both sexes in mice and in female rats. OSHA believes that because of the clear tumor response, and quality of the studies, the NTP studies provide the best data for quantitative cancer risk assessment. OSHA concludes from these studies that MC causes cancer in two species of test animals by the inhalation route, and that a clear dose-response has been demonstrated.

### 3. Epidemiological Studies

Epidemiological studies of occupational exposure to MC have been conducted in the manufacturing of triacetate fibers, photographic film production, and the manufacturing of paint and varnish. Those studies were reviewed by OSHA in the preamble to the proposed rule [56 FR 57075] and are summarized and updated in this document. In addition, an epidemiological study of MC exposure and astrocytic brain cancer is reviewed in this text.

*a. Studies of triacetate fiber production workers.* Ott et al. [Ex. 7-76] performed a retrospective cohort study using a cellulose diacetate and triacetate plant in Rock Hill, South Carolina to examine the effects of MC on a working population. In particular, Ott et al. evaluated the effects that were possibly mediated through the metabolism of MC to carboxyhemoglobin. Employees at this plant had MC exposures close to OSHA's time weighted average (TWA) permissible exposure limit (PEL) of 500 ppm. Ott et al. used workers in a plant in Narrows, Virginia as a comparison population because it had operations similar to those at the Rock Hill plant, but did not use MC. In this study, Ott et al. compared the number of deaths within the exposed cohort with the United States population and the Narrows, Virginia referent group. Ott et

al. observed that the overall mortality of the cohort was comparable to that of the age, sex, and race-matched U.S. population. Comparing exposed and referent cohorts, statistical differences in risk were observed in white men for "all causes" (risk ratio=2.2,  $p<0.01$ ), "diseases of the circulatory system" (risk ratio=2.2,  $p<0.5$ ), and "ischemic heart disease" (risk ratio=3.1,  $p<0.05$ ).

In interpreting the results of this study, Ott noted that there may have been differences in hiring practices in the two plants which could have contributed to the observed differences in mortality. In their conclusion, Ott et al. stated that a healthy worker effect (HWE) and the low power of their study did not permit them to dismiss the possibility of increased health risks within the working population exposed to MC.

Dr. Mirer of UAW testified [Tr. 1896-6, 9/24/92] that there is some evidence that there is excess work-related heart disease mortality in epidemiological studies that have observed SMRs greater than 80% for ischemic heart disease or any other cardiovascular disease. Furthermore, when the MC epidemiological studies are looked at together, there is evidence, although limited, that MC exposure has an effect on cardiovascular mortality.

On the other hand, Kodak [Ex. 91D] questioned the appropriateness of the referent population in the Rock Hill study, alleging that the SMR for ischemic heart disease in the referent population was unusually low, and that this fact, rather than an effect of MC exposure, caused the observed differences in ischemic heart disease rates.

In contrast, NIOSH considered the Rock Hill study to be suggestive of an effect of MC on risk of cardiac disease. According to NIOSH [Tr. 879, 9/21/92] the Ott study did not use appropriate analytic techniques that would allow the acute effects of MC on cardiac disease risk to be examined. Furthermore, NIOSH suggested [Tr. 969, 9/21/92] that future epidemiological studies should examine risks from MC exposure during the period when employees are actively working.

In an update to the Rock Hill study, Lanes et al. followed the Ott et al. cohort through September 1986 [Ex. 7-260] and December 1990 [Ex. 106]. Lanes et al. used the population of York County, South Carolina as the comparison group. Statistically significant excess mortality was observed for cancer of the liver and biliary passages (SMR=5.75, CI:1.82-13.78) in the study group. Excess mortality was also observed for buccal cavity and pharynx cancer

(SMR=2.31, 95% CI:0.39-7.60) and melanoma (SMR=2.28, CI:0.38-7.51), although mortality from these causes did not reach statistical significance. No excess mortality was observed for ischemic heart disease (SMR=0.90, CI:0.62-1.27).

Examination of the liver and biliary cancers indicated that the workers had ten or more years of employment and at least 20 years since first employment (4 observed v. 0.35 expected). Three of the four employees who died from liver/biliary cancer had tumor sites in the intrahepatic and common bile duct, common bile duct, and ampulla of Vater. Approximate durations of employment for these three cases were 28 years, 20 years, and less than one year. No medical record for the third case could be obtained. However, an autopsy report indicated adenocarcinoma of the liver for this case. To estimate the expected number of biliary cancer deaths, Lanes et al. used Surveillance, Epidemiology, and End Results (SEER) mortality rates of the continental United States. The computed risk estimate, based on 0.15 cases expected, was SMR=20 (95% CI:5.2-56.0).

The authors hypothesized that the biliary duct cancer cases may have been due to factors such as oral contraceptive use, gallstones, or ulcerative colitis. However, it appeared that medical records showed no indication of gallstones or ulcerative colitis in workers who died of biliary cancer. Moreover, although these factors were not specifically controlled for, there is no reason to believe the rates of these factors would be different in the exposed cohort compared to the general U.S. population.

Lanes et al. updated their study through December 31, 1990 [Ex. 106] using the National Death Index and focused on mortality from pancreatic cancer, biliary and liver cancer, and ischemic heart disease. Lanes et al. ascertained fifty more death certificates from the end of the last follow-up period on September 1, 1986. As before, York County, South Carolina was used as the comparison population.

The overall SMR from all causes of death was 0.90, and for malignant neoplasms, the SMR was 0.82. In this follow-up, the SMR for liver and biliary cancer dropped from 5.75 to 2.98 (95% CI:0.81-7.63). No additional deaths from biliary or liver cancer were observed. In the original and updated studies combined, four deaths from biliary/liver cancer were observed and 0.64 were expected. Using a Poisson distribution, Lanes et al. calculated the probability of failing to observe any liver/biliary

cancer deaths in this update if the "true" value of the SMR for liver/biliary cancer was 5.75 (from the previous study) and then expecting 3.68 deaths in this follow-up ( $0.64 \times 5.75$ ). They estimated the probability that this update would have no observed biliary/liver cancer deaths if the true SMR were 5.75, as  $e^{-3.68} = 0.025$ . On the other hand, if MC had no effect on liver and biliary cancer mortality, Lanes et al. estimated that the probability of observing zero deaths would have been 0.527 ( $e^{-0.64}$ ). Lanes et al. used the likelihood ratio ( $0.527/0.025 = 21.08$ ) to compare these two hypotheses. The authors concluded that the null hypothesis that the SMR=1.0 was 21 times more probable than the hypothesis that the SMR=5.75.

Because of the small number of cases involved and the instability of the numbers generated in this type of statistical analysis, OSHA believes that this study, overall, is suggestive (but not definitive) of an association between occupational exposure to MC and elevation of human cancer risk. Furthermore, the Agency has determined that the study results are not inconsistent with the results of the NTP cancer bioassay.

Hoechst-Celanese [Ex. 19-65, pp. 6-8; Ex. 19-19] was concerned that OSHA considered the incidence of biliary cancer as evidence of a positive effect. They argued that the reported excess in biliary tract cancer did not support the conclusion that MC exposure is associated with an increased risk of cancer. Specifically, they noted that,

(1) Biliary cancers have not been reported in any of the animal cancer studies of MC; (2) no statistically significant increase in biliary cancers was seen in the Cumberland study (described below); (3) no statistically significant excess in biliary cancers was reported in the Kodak studies (described below); (4) It was unlikely that MC could have been responsible for the biliary tract cancer observed in one employee who had been exposed to MC for less than one year; and (5) the Rock Hill study did not control for other chemical exposures.

Comments by the Halogenated Solvents Industry Alliance (HSIA) [Ex. 19-45, p. 47] were in accord with those of Hoechst-Celanese.

Dr. Shy, on behalf of Kodak, asserted [Tr. 1303, 9/22/92; Ex. 91F] that MC exposure failed to meet Bradford Hill's criteria for causality (e.g., biological plausibility, dose-response, and consistency) for producing biliary tract cancer. Dr. Shy acknowledged that animal bioassays have demonstrated liver tumors from MC exposure, but he noted that there is no evidence in humans that liver and biliary tract

cancers have the same etiology.

Furthermore, Dr. Shy argued that,

(1) the results from the Lanes study is not supported by *in vitro* or pharmacokinetic studies.

(2) a dose-response relationship could not be determined from the Lanes study because there were no direct measurements of worker exposure to MC.

(3) the observed association between MC exposure and liver/biliary cancer was an isolated finding and the existence of a causal relationship could not be concluded.

(4) the excess biliary tract cancer in the Lanes study was not consistent with the other three epidemiological studies (Hearne, 1987, 1990, 1992; Hearne, 1992; Gibbs, 1992).

Dr. Shy did recognize that there was a strong association between MC exposure and biliary tract cancer in the Lanes study (SMR=20). Moreover, the 20 year time interval between first exposure and death from biliary tract cancer provided evidence that "exposure preceded cancer with an appropriate interval for induction of the tumor [Ex. 91F]."

OSHA disagrees with the conclusions reached by Dr. Shy. The Agency believes that the risks of biliary cancer observed in these studies is consistent with risks derived from its pharmacokinetic analysis (see the Quantitative Risk Assessment, Section VI). Since the occupational exposures in these studies are likely to have been among the highest in any of the epidemiologic cohorts, there is no evidence that the increased biliary/liver cancer result is inconsistent with other reported epidemiological findings. Regarding the biological plausibility, the Agency notes that human biliary cells appear to contain high concentrations of the mRNA for GST (the enzyme many investigators believe to be responsible for MC-induced carcinogenesis) [Exs. 124 and 124A]. Although this requires more investigation to determine if there is a direct relationship, OSHA believes there is a plausible mechanistic argument for MC causality in human biliary tract cancers. The Agency agrees with Dr. Shy, however, that the lack of dose-response data and the small number of cases in this cohort limit the strength of conclusions that can be drawn from this study. After weighing these considerations, the Agency has determined that there is suggestive evidence of a causal role for MC in these cases of biliary cancer.

Gibbs et al. conducted a study of another cellulose acetate and triacetate fibers plant in Cumberland, Maryland [Ex. 54] to evaluate the possible

relationship between MC exposure and biliary/liver cancer. This plant, which ceased to operate in 1982, had operations similar to the plant in Rock Hill, and it was assumed to have had similar MC exposure levels as well. However, exposure measurements were not submitted for the Cumberland plant and it is unknown whether the Cumberland employees experienced the same exposures as their Rock Hill counterparts.

The Gibbs study investigated the mortality of 3,211 workers who were employed at this plant on or after January 1970. There were 2,187 men and 1,024 women in the cohort. Most of the workers in the cohort were hired prior to 1979 (2,566 total). The study population was divided into three subcohorts based on their estimated exposure to MC: 1) 834 men and 146 women in the "high exposure" group (estimated to be 350-700 ppm), 2) 1095 men and 832 women in the "low but never high exposure" group (estimated to be 50-100 ppm), and 3) 256 men and 46 women in the "no exposure" group. This cohort was followed through December 1989. The observed mortality was compared to expected death rates for Allegany County, Maryland (where the plant was located and where most of the cohort deaths occurred), the State of Maryland, and the United States.

The author of this study believed that the county rates were the most appropriate to use because the city of Cumberland is located in a rural area of Maryland and the state rates may have been influenced by rates in large urban areas such as Baltimore. In addition, local rates tend to adjust for social, economic, ethnic, and cultural factors which may be related to disease risk, access to medical care, etc. However, if the fiber plant was the major employer in this rural area, then county rates may reflect the cohort's mortality rather than the background risk, in which case, state rates or U.S. population rates would be more appropriate. The overall mortality rate for the high MC-exposed group was below the expected rates for Allegany County, Maryland, and the U.S. population.

As in the Rock Hill study, mortality from biliary tract cancer was observed in the Cumberland study, although no statistically significant elevated incidence of biliary cancer was found (two cases of biliary tract cancer were observed). In the high exposure group, there was one death (1.24 expected with Allegany rates (SMR=80.5) and 1.42 expected with Maryland rates (SMR=70.4)). In the low MC-exposed group, there was also one death from biliary/liver cancer. For the high MC-

exposed subcohort, Gibbs et al. estimated SMRs of 80.4, 70.3, and 75.1 when comparisons were made with Allegany County, Maryland, and U.S. rates, respectively. In the low MC-exposed subcohort, the SMRs using Allegany and Maryland rates were 75.4 and 76.4, respectively. This cohort should be followed for a longer period of time to help clarify the suggested association between MC exposure and biliary cancer observed in the Rock Hill cohort.

Statistically significant excess mortality was also observed from prostate, uterine, and cervical cancers, although these also represented small numbers of cases: 13, 2, and 1, respectively.

The excess of prostate cancer in the Gibbs et al. study suggested an exposure-response relationship (3 deaths in no MC-exposure group, 9 in low MC-exposure group, and 13 in high MC-exposure group). According to Gibbs et al. and Shy [Tr. 1303, 9/22/92; Exs. 19–64, 91F], this response may have been related to other chemical exposures (occupational or non-occupational). In support of this hypothesis, no other epidemiological or animal studies of MC exposure have suggested a relationship between prostate cancer and MC. Hoechst-Celanese [Ex. 19–65, pp. 10–12; Ex. 91D, p. 12] cautioned OSHA not to overinterpret the excess of prostate cancer in the Cumberland study for the following reasons:

(1) of all the epidemiological studies, only the Cumberland study has shown an excess of prostate cancer; (2) of the thirteen high subcohort men who died of prostate cancer, twelve worked in the extrusion area of the Cumberland plant before methylene chloride was used as a solvent in cellulose triacetate fiber production. Thus, these men may have had longer exposure to other chemicals; (3) the study did not control for other personal risk factors; (4) Gibbs reported an increased incidence of prostate cancer elsewhere in the textile industry; and (5) the large number of statistical tests may have increased the probability of finding the death rate of a specific cause to be elevated or depressed.

OSHA believes that the increased risk of prostate cancer should be noted as a possible positive effect of MC exposure on cancer risk, particularly considering the exposure-response relationship. However, because of potential confounding factors and lack of corroborating findings in other studies, OSHA believes this is suggestive rather than conclusive evidence of a human carcinogenic effect.

*b. Studies of film production workers.* In their original study of film production workers, Friedlander et al.

[Ex. 4–27] conducted both a proportionate mortality study and a retrospective mortality cohort study to determine if workers exposed to MC experienced an increased risk for specific causes of mortality. The cohort in these studies consisted of workers who worked in any department in film production that used MC as its primary solvent for approximately thirty years. The cohort was followed through 1976.

Proportionate mortality analysis for those workers ever employed in the study area versus a comparison group of workers in other Kodak Park departments produced a proportionate mortality ratio (PMR) of 143.88 for liver (intrahepatic ducts-primary) cancer. For ischemic heart disease, Friedlander et al. calculated a PMR of 94.74. No statistically significant differences were observed at  $p \leq 0.05$ .

For the cohort mortality study, Friedlander et al. used rates from the 1964–70 hourly males age group exposed to MC in the film department and the other Kodak Park departments for internal comparison. Mortality rates for New York State, excluding New York City, males age group were used for external comparisons.

Forty-five deaths from circulatory diseases were observed in the MC-exposed cohort versus 38.5 expected in the Kodak Park referent group. Also, 6 deaths from respiratory diseases were reported in the MC-exposed group versus 3.2 expected for the Kodak Park comparison group. No liver deaths were observed in this cohort. Thirty-three deaths from ischemic heart disease were observed in this cohort compared with 28.7 expected in the Kodak Park population. None of these observed differences in mortality reached statistical significance.

Hearne et al. conducted several updates to the cohort study involving MC exposure and mortality among workers in film production areas at the Kodak plant in Rochester, New York [Exs. 7–122, 7–163, 49 A–1]. In the first update, the study cohort was followed through 1983. Two referent groups were utilized in this study: the general population of upstate New York men, excluding New York City, and Kodak Park employees.

No statistically significant findings were observed for any cause of death. However, Hearne et al. did find a relatively large number (8 observed) of pancreatic cancer deaths compared with the New York State (3.2 expected) and Kodak (3.1 expected) populations. This observation did not achieve statistical significance and a dose-response relationship was not observed when

Hearne et al. considered latency and dose.

Hearne et al. then updated this study through 1988 [Ex. 7–163] and 1990 [Ex. 49 A–2]. In the 1988 update, nonsignificant deficits in observed-expected ratios for lung and liver cancer were found. Also, overall mortality from 1964 to 1988 was significantly less than in both referent groups. Since 1986, the number of pancreatic cancer deaths remained the same. As before, dose-response analysis showed no statistically significant pattern when latency or dose were considered.

The 1990 update showed that deaths due to liver cancer, lung cancer, and ischemic heart disease were below the expected numbers in both referent groups. Also, no additional pancreatic cancer deaths were observed in this second update. Since the start of the follow-up, Hearne et al. observed 8 deaths from pancreatic cancer compared with 4.5 expected (SMR = 1.78,  $p = 0.17$ ).

Hearne et al. [Ex. 49 A–1] conducted a second Kodak cohort study involving workers in cellulose triacetate preparation and film base manufacturing between 1946 and 1970. Hearne et al. addressed the potential selection bias in the 1964–70 Kodak cohort by including only workers exposed primarily to MC after it was introduced in these areas and making the study more complete by adding workers in the Dope Department, which prepares the viscous cellulose triacetate mixture used in the film base coating, and the Distilling Department, which redistills and reblends solvents recovered from the coating operations.

The 1,311 men in the cohort were followed through 1990. An occupational control group could not be formed because death rates for Kodak employees before 1964 were unavailable. Instead, male residents of upstate New York living outside of the five New York City counties were used.

Hearne et al. combined exposures by job and time period with occupational history information to produce a career exposure estimate for each individual in the study for dose-response analyses. The mean career individual exposure was approximately 40 ppm for 17 years and the average interval between first exposure and end of follow-up was about 32 years.

Total mortality for this cohort was 22% below the expected mortality (statistically significant). Circulatory diseases and ischemic heart disease mortality were also statistically significantly below expectation. For lung cancer there were 22 deaths (28.7 expected) and for liver/biliary cancer

there was one death (1.5 expected). Hearne et al. found that the number of pancreatic cancer deaths observed (4) was similar to the expected number (4.4). In this cohort, the number of observed deaths was greater than expected for diseases of the colon/rectum (13 observed v. 10.8 expected), brain (5 v. 2.3), and for leukemia (7 v. 3.4), but were not statistically significant.

Hearne et al. concluded that the findings in the 1964–70 cohort were consistent with the 1946–70 cohort: mortality from all causes, cancer (including lung and liver malignancies), and ischemic heart disease was lower than expected. Also, since the number of observed pancreatic cancer deaths in this cohort was similar to the expected number, Hearne et al. believed that this provided further evidence that the earlier finding of an excess of pancreatic cancer in the 1964–70 cohort was due to chance or to factors other than MC exposure.

Kodak [Tr. 1287–88, 9/22/92] also investigated the risk of adverse health effects during active occupational exposure to MC, as suggested by NIOSH [Tr. 970, 9/21/92]. Using person-years of active employment only in their analysis, Hearne observed 27 deaths (36 were expected in the internal Kodak reference group) from ischemic heart disease in the 1964–70 Kodak cohort; in the 1946–70 cohort, Kodak recorded 33 deaths compared with 43 expected in the New York State comparison population.

NIOSH testified [Tr. 877–83, 9/21/92] that the healthy worker effect (HWE) could have obscured any excess mortality from ischemic heart disease caused by MC exposure. NIOSH has stated that the HWE may be particularly strong for cardiovascular diseases.

The HWE is likely to be less of a factor when occupational comparison groups are used. Kodak's use of the Kodak Park employees as a comparison group should reduce the HWE in its studies. However, there are two potential problems with using occupational comparison groups in this instance:

- (1) Cancer rates are more stable in larger populations, so comparison with state and national rates may be more appropriate.

- (2) Due to the volume of MC used in the Kodak plant, the occupational comparison group may be exposed to air- or water-borne environmental concentrations of MC which could obscure the impact of occupational exposure to MC on cancer incidence.

*c. Study of workers in paint and varnish manufacturing.* The NPCA

submitted to the record an epidemiological study of employees who worked for at least one year in the manufacture of paint or varnish [Ex. 10–29B]. OSHA's review of this study was published in the proposed rule [56 FR 57077]. Although no statistically significant excess of mortality was reported, OSHA noted that there were 4 pancreatic cancers (1.93 expected) and 15 cancers of digestive organs and peritoneum (10.66 expected) among MC-exposed workers.

*d. Astrocytic brain cancer among workers in electronic equipment production and repair.* In its March 11, 1994 Notice of Limited Reopening of the Rulemaking Record, OSHA solicited comments on a case-control study submitted to the Agency by the National Cancer Institute (NCI) [Exs. 112 and 113].

Heineman et al. conducted a case-control study to examine the potential association between brain cancer and exposure to organic solvents as a group and six chlorinated aliphatic hydrocarbons (CAHs) including MC. Cases were defined as white males who died from brain or other central nervous system tumors in southern Louisiana, northern New Jersey, and Philadelphia, Pennsylvania. Controls were randomly selected from death certificates and included white males who died of causes other than brain tumors, cerebrovascular diseases, epilepsy, suicide, and homicide. Controls were frequency-matched to cases by age, year of death, and geographic area.

Four-digit Standard Industrial Classification (SIC) and 4-digit Standard Occupational Classification (SOC) codes were employed to code occupational histories of study subjects. These codes linked work histories to job-exposure matrices which “characterized likely exposure to the six CAHs and to organic solvents” [Ex. 112]. Gomez et al. [Ex. 112] used an algorithm to assign estimates of probability and intensity of exposure to each industry/occupation combination in subjects' work histories. As noted by Gomez et al., these estimates were based on “occupation alone, industry alone, or both occupation and industry, depending on the specificity of the exposure environment that could be inferred from the occupational (SOC) code.”

The following surrogate measures of dose, for each substance, were used to summarize “likely” exposure histories for each study subject: duration of employment in occupation/industry combinations considered exposed, a cumulative exposure score, and “average” intensity of exposure. Odds ratios were calculated for exposure

intensity categories to refrain from using weights. These categories did not include duration in jobs with lower intensity for subjects with high or medium intensity jobs. In their statistical analyses, Heineman et al. controlled for age, geographic area, and employment in electronics-related occupations/industries.

Astrocytic brain cancer was not found to be associated with “ever” being exposed to organic solvents as a group or to any of the six CAHs examined in this study. However, as probability of exposure to organic solvents as a group, and MC in particular, increased, the risk of brain cancer increased (chi-squared statistics for trend for organic solvents and MC were 1.93 and 2.29 ( $p<0.05$ ), respectively). For MC there was a 2.4-fold increase in risk for subjects with a high probability of exposure (confidence interval=1.0–5.9).

Risk of brain cancer significantly increased with duration of exposure for subjects with high probabilities of MC exposure (OR=6.1; CI=1.1–43.8). Heineman et al. found that, in the high probability of MC exposure category, risk significantly increased with duration (chi for trend=2.58,  $p<0.01$ ). Similar results were seen for organic solvents and methyl chloroform for all probabilities combined (chi-squared statistics for trend were 2.35 ( $p<0.01$ ) and 1.87 ( $p<0.05$ ), respectively).

Lagging exposure by 10 years produced findings analogous to those noted above. Higher risks and a sharper increase with duration was observed for organic solvents when exposure was lagged by 20 years (all probabilities: 2–20 years, OR=1.3 (95% CI=0.9–2.0); 21+ years, OR=2.8 (1.1–3.7);  $p$  for trend=0.006; high probability: 2–20 years, OR=1.2 (95% CI=0.7–1.9); 21+ years, OR=3.1 (1.3–7.4),  $p=0.009$ ).

Subjects with a high probability of MC exposure experienced a statistically significant increased risk as the cumulative exposure score increased (chi-squared statistics for trend=2.18,  $p<0.05$ ). However, risk did not increase monotonically with cumulative exposure.

Lagging exposure 20 years supported the odds ratios and the trends for organic solvents, particularly in men with a high probability of exposure (low cumulative score: OR=1.1 (95% CI=0.5–2.3); medium: OR=1.4 (0.8–2.5); high: OR=2.2 (1.0–4.5);  $p$  for trend=0.02). Few individuals had high cumulative scores when exposure was lagged 20 years for the individual CAHs.

Compared with jobs with medium or low intensity exposures to organic solvents and all six CAHs, risk of brain cancer was higher for subjects who



worked in jobs with high intensity exposures. Brain cancer was associated most strongly, and increased with probability of exposure, among subjects who worked 20 or more years with high intensity exposure to MC (all probabilities: OR=6.7, CI=1.3–47.4; high probability: OR=8.8, CI=1.0–200.0).

Since many subjects were determined to have been exposed to more than one of the CAHs, sometimes even in the same job, Heineman et al. used logistic regression to examine, simultaneously, the effects of MC, carbon tetrachloride, tetrachloroethylene, and trichloroethylene, controlling for age, geographic area, and employment in electronics-related occupations/industries. MC was the only substance to show a statistically significant increase in risk as the probability of exposure increased (low: OR=0.9, CI=0.5–1.6); medium: OR=1.4, CI=0.6–3.1; high: OR=2.4, CI=0.9–6.4; chi-squared statistics for trend=2.08,  $p<0.05$ ). Risks associated with MC increased when adjustments for exposure to the other agents were made. In addition, subjects employed for 20 years or more in jobs with high average intensity MC exposure showed an eight-fold excess of brain cancer (OR=8.5, CI=1.3–55.5), taking all probabilities into consideration.

Among the six CAHs examined in this study Heineman et al. found the strongest association between brain cancer and MC-exposure, for which relative risks rose with probability, duration, and average intensity of exposure, though not with the cumulative exposure index.

According to Heineman et al., the major weakness of this study was not having direct information on exposure to solvents. Next-of-kin data, poor specificity of some work histories for specific solvents, and the interchangeability of solvents may have resulted in misclassification of individuals with respect to any of the exposure measurements used in this study. However, Heineman et al. pointed out that the potential sources of error probably did not significantly bias risk estimates away from the null or generate the observed trends.

Another limitation of this study, pointed out by Heineman et al., was that over one-third of the next-of-kin of eligible cases and controls were not interviewed. According to Heineman et al., this could have artificially created the associations seen in this study “only by underrepresenting cases who were unexposed, and/or controls who were exposed, to solvents in general, and MC in particular” [Ex. 113]. Heineman further remarked that differential

misclassification was probably not a problem in this study because occupational histories came from next-of-kin of both cases and controls.

In light of the limitations of this study, however, Heineman et al. commented that the consistency of exposure-response trends for MC was surprising and suggestive. Moreover, Heineman et al. believed that the trends and consistency of the associations between brain cancer and MC could not be explained by chance alone.

Several commenters [Exs. 115–1, 115–31, 115–32, 115–36] indicated that Heineman et al. relied too heavily on next-of-kin information. Information provided by next-of-kin concerning jobs held, job descriptions, dates of employment, and hours worked per week may be flawed with recall bias. Next-of-kin may not be able to accurately recall job-related information, especially for jobs held early in life. If next-of-kin for cases or controls had better recall than the other group, differential misclassification could occur. HSIA [Ex. 115–36] stated that even small differences in error rates between cases and controls could produce false associations. Both HSIA and NIOSH [Ex. 115–31] agreed that this indirect source of exposure information was likely to produce some degree of misclassification. However, NIOSH noted that misclassification “is a typical problem in population based case-control studies of this type [Ex. 115–31]” and that this misclassification could also explain the fact that no associations were found between brain cancer and the cumulative exposure score.

Organization Resources Counselors (ORC) [Ex. 115–2] and Abbott Laboratories [Ex. 115–30] were concerned that the lack of exposure verification made this NCI study unreliable for setting MC exposure limits. ORC stated that exposure values were assigned to all SIC and SOC codes, and not developed based on job history information, which would have given the study more validity. Kodak also expressed some concern regarding this study due to lack of accurate records of past exposures, reliance on expert judgement to a large degree, use of next-of-kin to determine potential exposure, and undocumented qualifications of those making judgements concerning the different occupations and industries involved. In addition, Kodak felt that the exposure data were “at best, unsubstantiated semi-qualitative judgements of likelihood and intensity of exposure [Ex. 115–1].” Organization Resources Counselors [Ex. 115–2] and Abbott Laboratories [Ex. 115–30]

asserted that it was impossible to tell if those who died of cancer had been exposed to MC because there was no exposure verification. Vulcan Chemicals [Ex. 115–32] criticized the investigators for not going to work sites and determining the actual magnitude of exposure to the CAHs. HSIA [Ex. 115–36] argued that “concordance of proxy reports with actual work histories may range from 0–50% for decedents’ first jobs and from 50–70% for last jobs.” OSHA believes that exposure verification would have increased the validity of the findings of this study. However, lack of exposure verification does not nullify the results of the study. The Agency believes that the associations observed are suggestive of a human carcinogenic effect of MC.

Another issue that Kodak [Ex. 115–1] and Vulcan [Ex. 115–32] emphasized was the possible exposure to other chemicals or sources of potential human carcinogens, such as ionizing radiation, electromagnetic fields, smoking history, and place of residence. Vulcan [Ex. 115–32] noted that there may have been selection bias in this study because of the large ratio of astrocytic brain cancer tumors to the total number of brain tumors. Although they offered no explanation of how this selection bias would operate, Vulcan did suggest that this issue should be investigated further.

Vulcan was also concerned that the matching of controls and cases with respect to occupations and socioeconomic status may be inadequate. In particular, Vulcan criticized the Heineman study for not presenting the occupations of the control group and for not matching the socioeconomic status of the two groups. Similarly, Kodak [Ex. 115–1] stated that some adjustment should have been made in order to match across educational levels.

Kodak [Ex. 115–1] also believed that the estimates of trends observed in this study could have been affected, if workers in the longest duration or the higher probability of exposure categories had longer dates of employment, worked in more stable industries, and had better health benefits, better access to medical care, and more sophisticated diagnostic procedures. OSHA believes that there is no evidence that this is the case in this study.

HSIA [Ex. 115–36] criticized the methodology for assessing the number of industries with exposures to CAHs. HSIA argued that Gomez et al. did not fully explain how they determined that workplaces in the specific SICs would have CAH exposures. According to HSIA, Gomez et al. reported inaccurate

information regarding industry use of MC. HSIA cited EPA's "Toxic Air Pollutant/Source Crosswalk, A Screening Tool for Locating Possible Sources Emitting Toxic Air Pollutants (EPA-450/4-87-023A, Dec. 1987)" which revealed a higher number of SIC codes using MC. In conclusion, HSIA asserted that Gomez et al.'s "exposure scenario" was incorrect.

Several commenters [Exs. 115-1, 115-31, 115-36] argued that the Heineman et al. study should only be considered a hypothesis-generating study and should not be used to adjust the PEL.

OSHA agrees with NIOSH that the Heineman et al. study was well-conducted because there was a systematic attempt to estimate exposure by work experience. Furthermore, there was a remarkably high correlation between exposure to MC and brain tumors. OSHA concludes that the results from this study strongly suggest a possible association between MC and brain cancer. However, in the absence of quantified exposure data for these workers, it remains relatively speculative to attempt to estimate a quantitative dose-response relationship. Therefore, OSHA concludes that the risk estimate based on the animal data is the best available and accordingly it retains that estimate for its significant risk analysis.

*e. Summary of epidemiological studies. Considered as a whole, the available epidemiologic evidence did not demonstrate a strong, statistically significant cancer risk associated with occupational exposures to MC. However, the positive trend for biliary tract/liver cancer deaths, the association between occupational MC exposure and astrocytic brain cancer and the statistically significant excess prostate cancer results are suggestive of an association between MC exposure and cancer risk. In addition, the non-positive epidemiological studies summarized here are not of sufficient power to rule out the positive results from the animal studies. This issue is addressed further in the Quantitative Risk Assessment section of this document.*

In summary, the epidemiological results are suggestive of an association between occupational exposure to MC and elevated cancer risk which offers supporting evidence to the positive animal bioassay results.

#### 4. Conclusion

OSHA concludes from the mutagenicity, animal bioassay and human epidemiology data that MC causes cancer in test animals and that it is a potential occupational carcinogen.

The Agency has determined that, because of the quality of the studies, the clear dose-response relationship and the appropriateness of the route of administration, the NTP rodent bioassay data are the best available for quantitative cancer risk assessment.

OSHA also concludes that the epidemiology data, in some cases, suggest a positive association between human MC exposure and cancer incidence, but the dose-response relationships are not clear. The Agency has determined that the remaining epidemiology data (the non-positive studies) are not of sufficient power to rule out the results obtained in the animal bioassay data and that the animal data provide the best available data for quantitative risk assessment.

#### E. Other Toxic Responses

##### 1. Central Nervous System Toxicity

MC acts on the central nervous system (CNS) as a CNS depressant. CNS depression has been described in humans exposed to MC concentrations as low as 175 ppm (8-hour TWA). This depression in CNS activity was manifested as increased tiredness, decreased alertness and decreased vigilance. These effects could compromise worker safety by leading to an increased likelihood of accidents following MC exposure.

*a. Animal studies.* In the NPRM, OSHA reviewed two animal studies of MC CNS toxicity (briefly summarized below) and concluded that the CNS was potentially susceptible to reversible and irreversible effects due to MC exposure.

Savolainen et al. [Ex. 7-178] studied biochemical changes in the brains of rats exposed to MC. Rats were exposed to 500 ppm MC for 6 hr/d. On the fifth day, after 3 and 4 hours of exposure to MC, levels of acid proteinase in rat brains were significantly increased, but no change in brain RNA levels was reported. The authors suggested that the increase in acid proteinase may have been the result of increased levels of CO from metabolism of MC. OSHA believes that this study shows that MC can cause specific changes in the neurological system at a biochemical level. The Agency intends to monitor the scientific literature for additional developments on these effects, but has not used this information in setting the MC exposure limits because it is presently unclear how changes in acid proteinase are related to the observed CNS depressive effects of MC in humans.

Rosengren et al. [Ex. 7-56] looked at the effects of MC on glial cell marker proteins and DNA concentrations in gerbil brains after continuous exposure

to 210, 350 or 700 ppm MC. Because of high mortality in the 2 higher doses, no data were collected at 700 ppm and exposure was terminated after 10 weeks at 350 ppm. Exposure to 210 ppm was continued for three months. Exposure to MC was followed by four months of no exposure before animals were examined for irreversible CNS effects. The authors found increased levels of glial cell marker proteins in the frontal cerebral cortex and sensory motor cortex after exposure to 350 ppm MC. These findings are consistent with glial cell hypertrophy or glial cell proliferation. Levels of DNA were decreased in the hippocampus of gerbils exposed to both 210 and 350 ppm and in the cerebellar hemispheres after 350 ppm MC. Decreased DNA concentrations indicate decreased cell density resulting from cell death or inhibition of DNA synthesis.

The neurotoxic mechanism of action of MC in gerbil brains is not understood. However, since the metabolism of MC to CO was determined to be saturated at both 210 and 350 ppm (COHb levels were equivalent at both exposure concentrations), the changes in glial cell proteins and DNA concentrations was attributed to either a direct effect of MC or an effect of a metabolite of the GST pathway. Although this study describes biochemical changes in the CNS subsequent to MC exposure, the high mortality of the experimental animals and the lack of MC toxicity data in the gerbil make it difficult to determine the significance of this study for extrapolation to other species. It is also unclear how these effects would relate to CNS depression observed in humans after MC exposure. In addition, continuous exposure to MC has been shown in other experimental situations [Exs. 7-14 and 7-130] to elicit more severe health effects than exposure to similar or higher concentrations when the animals are allowed a recovery period (for example, 6 hours' exposure per day). Exposure on a 6 or 8-hour per day schedule is also more like occupational exposure scenarios and therefore those experiments are generally easier to interpret when assessing risk to workers.

In summary, OSHA believes that the rat and gerbil data described above shows that MC can cause specific changes in the neurological system at a biochemical level. The Agency intends to monitor the scientific literature for additional developments on these effects to determine if these types of effects have implications for human CNS risks.

*b. Human studies.* The CNS depressant effects of MC have been well described in the literature [Exs. 7-4, 7-153, 7-154, 7-160, 7-175, 7-182, 7-183, 7-184]. MC causes CNS depression which is characterized by tiredness, difficulty in maintaining concentration, decreased task vigilance, dizziness, headaches, and, at high concentrations, loss of consciousness and death. Accidental human overexposures to MC [Exs. 7-18, 7-19] (for example, at concentrations greater than 10,000 ppm) have resulted in narcosis and death. CNS depression has been described after humans were exposed to experimental MC concentrations as low as 200 ppm [Ex. 7-175] and occupational concentrations as low as 175 ppm [Ex. 7-153].

*i. Experimental studies.* CNS depression was detected in human subjects exposed to MC at concentrations as low as 200 ppm for 4 hours or 300 ppm for 1.5 hours [Exs. 7-4, 7-160, 7-175, 7-182 and 7-184]. In these experiments, which measured subtle CNS depression (such as dual task performance and visual evoked response), it was not possible to determine a no observed effect level (NOEL), because the lowest experimental concentration used (200 ppm) elicited CNS effects. Since a NOEL was not determined for the CNS effects of MC, those effects may occur at lower exposures or after exposure for shorter durations.

The HSIA questioned whether bias was introduced into the results of these studies by inadequate procedures to establish a "double blind." This criticism raises a legitimate concern about the validity of the study. However, since Putz et al. did not describe the blinding procedures used in their experiments, the Agency concludes that there is not enough evidence publicly available to make the conclusion that the study is biased. OSHA believes that these studies were well conducted and is relying on the quality of the studies overall as evidence of the validity of the results. Absent evidence demonstrating the inadequacy of the blinding procedures, OSHA has determined that these studies show that MC can cause mild CNS depression in humans exposed at concentrations as low as 200 ppm.

NIOSH expressed concern regarding the potential for neurobehavioral impairment (expressed as CNS depression) at lower exposures and shorter durations, particularly in relation to the setting of a STEL for MC [Exs. 23-18 and 94]. In order to assess the potential impact of the CNS effects of MC, NIOSH looked at data gathered

from several studies and compared breath concentrations of MC (as a surrogate for brain tissue MC concentrations) at different ambient exposure levels with the CNS depression described by Putz et al. [Ex. 7-175]. NIOSH concluded that:

At the proposed STEL of 125 ppm, increased uptake of MC in active workers may place them in the breath concentration range associated with mild neurobehavioral impairment. Although there are insufficient data to draw firm conclusions, extrapolation from existing studies suggests that the proposed STEL of 125 ppm may not fully protect physically active workers from CNS impairment. Therefore, a lower STEL should be considered, if feasible.

In response to concerns raised by NIOSH, the HSIA [Ex. 105] noted that NIOSH's analysis of breath MC concentration versus neurobehavioral impairment "seemed highly speculative." HSIA emphasized that the exposures which produced the reported neurobehavioral effects were observed only after 2 to 4 hours of exposure and that the effects were observed only when difficult tasks were measured.

To support their position, the HSIA asked Mr. Richard Reitz to use a PBPK model to estimate the concentration of MC in brain tissue. This analysis [Ex. 105] indicated that at exposures of 200 ppm for 15 minutes with persons exercising at 50 watts, the brain concentration of MC would be predicted to be similar to that observed in the Putz et al. study for subjects engaged in "light activity" for 2 hours at 200 ppm MC, which did not produce measurable CNS depression. (Putz et al. did not detect CNS depression in subjects exposed to 200 ppm for 2 hours). The model also predicted that 15-minute exposures to 125 ppm while the subject was exercising at 50 watts would produce brain MC concentrations substantially less than that predicted for the 4 hour exposure to 200 ppm MC.

OSHA considered the PBPK analysis presented by the HSIA, but was concerned that there has been no experimental validation of the predicted brain MC concentrations or any evidence as to what MC concentration would produce detectable CNS depression. OSHA believes the primary value of both the NIOSH and HSIA analyses is in demonstrating the relative effect that exercise and duration of exposure is likely to have on brain (or breath) concentrations of MC. The PBPK analysis clearly demonstrates that increasing exercise level increases brain concentration of MC, which is consistent with the detected CNS depression. Workers engaged in strenuous activity while exposed to MC

should take special precautions, such as frequent breaks in fresh air, especially if dizziness or lightheadedness occurs.

Although OSHA found the PBPK model to be useful for demonstrating the interaction between exercise and brain concentration of MC, the Agency did not use the model quantitatively (for example, in determining the STEL). OSHA believes that the data suggest that there may be CNS effects at levels below those tested. There are no studies which directly address whether there are CNS effects after exposure to STEL concentrations of MC. To the extent that these effects occur, the STEL would not be protective. Mild and reversible CNS depression was detected at 200 ppm for 4 hours and 300 ppm for 1.5 hours. The Agency shares NIOSH's concern, based on extrapolation of breath MC concentrations, that the proposed STEL may not be adequately protective for physically-active workers.

OSHA concludes that there are clearly sufficient data to determine that a 125 ppm 15-minute STEL is needed to prevent a significant risk of material impairment to the CNS. Impairment of the CNS would also increase the risk from accidents. Measured data show risks at 200 ppm for four hours of exposure. A lower level at shorter duration is needed to avoid that risk. NIOSH's calculations show that for active workers a level lower than 125 ppm may be needed. However, because of feasibility concerns, which would be greater at lower levels and the suggestion that short duration of exposure (i.e., 15-minutes) may mitigate the effects, OSHA is retaining the proposed level, but will carefully monitor and follow up data to determine if this level eliminates significant risk.

*ii. Occupational exposure studies.* In the NPRM, OSHA summarized studies which it believed described a neuropathy associated with chronic occupational exposure to solvents. Weiss [Ex. 7-196] described the case of a 39-year old chemist who worked for 5 years with airborne concentrations of MC as high as 660 ppm to 3600 ppm in a room with poor ventilation. After 3 years of exposure, the worker developed neurological symptoms, characterized by restlessness, palpitations, forgetfulness, poor concentration, sleep disorders, and finally, acoustical delusions and optical hallucinations. No hepatic damage or cardiac toxicity was found. At the first appearance of symptoms, cessation of exposure produced an immediate cessation of symptoms. Later, longer and longer periods were required after termination of exposure in order to alleviate the

symptoms. The increasing persistence of symptoms is consistent with a diagnosis of toxic encephalosis.

Hanke et al. [Ex. 7-195] examined 32 floor tile setters who were exposed primarily to MC at concentrations from 400 to 5300 ppm for an average tenure of 7.7 years. Clinical examination of 14 of the workers who had neurological symptoms (headache, vertigo, sleep disturbance, digestive complaints and lapses in concentration and memory) revealed changes in the EEG patterns of the exposed workers which persisted over a weekend pause in exposure. These EEG changes were characteristic of a toxic encephalosis produced by chronic intoxication with a halogenated solvent (MC). The persistence of the EEG changes over the weekend break indicated a prolonged effect of MC exposure on EEG patterns. (Additional changes in the EEG found during exposure could be attributed to an acute effect of MC). Although these studies represent a small number of cases with very high chronic exposures, the evidence is suggestive of a relationship between chronic MC exposure and toxic encephalosis.

In a case study report, Barrowcliff et al. [Ex. 7-123] attributed cerebral damage in a case study to CO poisoning caused by exposure to MC. Axelsson [Ex. 7-150] has described an increased number of neuropsychiatric disorders among occupations with high solvent exposures.

In the NPRM, OSHA expressed the opinion that these studies, taken together, "provide suggestive evidence of a permanent toxicity [different from the observed reversible CNS depression] which may be the result of chronic exposure to MC." NIOSH stated that this assessment was too speculative and stated,

in the Hanke study, MC was apparently only one component of a solvent mixture and may not have been the only neurotoxic agent\* \* \* In addition, the observation interval of 2.5 days was not long enough to provide convincing evidence of irreversible effect, regardless of the active agent.

Upon reexamination of these studies, OSHA agrees with NIOSH [Ex. 19-46] that although a prolonged effect (over a weekend break in exposure) of MC on EEG patterns has been demonstrated, these studies do not support a determination that MC exposure is associated with irreversible brain damage in humans.

OSHA reviewed several other studies of occupational exposure to MC for evidence of CNS effects of MC. The first study was provided as an English translation of a Czechoslovakian paper by Kuzelova et al. [Ex. 7-26]. These

investigators examined workers in a film production plant who were exposed to MC concentrations from 29 to 4899 ppm. Several workers suffered frank MC intoxication and many workers showed signs of MC-induced CNS depression. Toxicity associated with chronic MC exposure was observed in workers exposed to MC for up to two years, but the authors recommended continuing studies of the long-term health effects.

OSHA believes that this study shows CNS depression in workers exposed to MC. The Agency agrees with the authors that this study was not sufficient to adequately characterize the long-term CNS health effects that may be induced by MC exposure.

Cherry et al. [Ex. 7-154] studied the effects of occupational exposure to MC at 28 to 175 ppm in two exposed populations. In a 1981 study, the authors found a marginal increase in self-reported neurological symptoms among exposed workers. This increase disappeared when an appropriate reference group was used for comparison. However, in a 1983 investigation, Cherry [Ex. 7-153] showed statistically significant increases in tiredness and deficits in reaction time and digit symbol substitution which correlated with MC in blood. Ambient MC exposures for this population ranged from 28 to 175 ppm for the full shift. This study demonstrated CNS effects due to occupational MC exposures below 200 ppm (the lowest dose which was administered in the experimental studies).

The HSIA [Ex. 105, p. 34] commented as follows:

Decades of experience with worker populations exposed even at levels up to the current 500 ppm TWA have provided no evidence that such workers have higher rates of accidents or other signs of significant neurobehavioral impairment.

To the contrary, OSHA believes that the occupational studies discussed above demonstrate that MC has an effect on the CNS at occupational exposure levels as low as 175 ppm.

The Agency believes that the 1983 study by Cherry shows that occupational exposure to MC concentrations below the former 8-hour TWA PEL of 500 ppm can produce detectable CNS effects. Although the 1981 study, which relied on self-report of neurological symptoms, did not demonstrate a CNS effect, the 1983 study examined more objective measures of CNS depression and correlated the observed effects with a direct measure of MC exposure. OSHA

believes that this study demonstrates that, although the CNS depression may be mild, it is demonstrable in occupational settings and at concentrations in the range of the STEL (although the exposures in this study were over an 8-hour work day). As described above, OSHA is sufficiently concerned about the potential for health effects at concentrations below the STEL of 125 ppm that it will continue to gather information and revisit this issue, if warranted.

## 2. Cardiac Toxicity

As described in the section on the metabolism of MC, MC is metabolized *in vivo* (in animals and humans) to CO and CO<sub>2</sub>. Cardiovascular stress has been observed after exposure to CO, so it is reasonable to suspect that similar health effects would be observed after exposure to MC (and metabolism to CO) [Ex. 7-73, 4-33]. Carbon monoxide successfully competes with oxygen and blocks the oxygen binding site on hemoglobin, producing carboxyhemoglobin (COHb) and reducing delivery of oxygen to the tissues. This reduces the oxygen supply to the heart itself, which can result in myocardial infarction (heart attack) [Ex. 4-33].

Generally, humans have a baseline level of COHb of less than 1% COHb due to the endogenous production of CO from normal metabolic processes. The measured level of COHb in the general non-smoking population is from 1% to 3% because of direct exposure to CO from combustion sources such as automobiles, etc. In smokers, COHb generally ranges from 2% to 10% because of the additional CO exposure during smoking. CO generated from exposure to MC would be additive to the COHb burden already experienced by an individual from direct exposure to CO. The cardiac health effects anticipated from exposure to MC itself or CO as the result of metabolism of MC are described below.

*a. Animal studies.* There is no evidence from animal studies in the MC rulemaking record that MC has a direct toxic effect on cardiac tissue. After lethal doses of MC, death has been primarily attributed to CNS and respiratory depression [Exs. 7-27, 7-28]. Also, chronic studies (in which COHb levels have been maintained at 10% and higher) [Exs. 7-3, 7-8, 7-14, 7-130, 7-151] have not shown direct cardiotoxicity.

Chlorinated solvents have been shown to sensitize the cardiac tissue to epinephrine-induced fatal cardiac arrhythmias [Ex. 7-226]. However, MC is less effective in sensitizing cardiac

tissue than other chlorinated analogues. MC caused sensitization of cardiac tissues only at doses well above doses which produce a narcotic effect. This finding indicates that compliance with an 8-hour TWA of 25 ppm MC would likely be sufficient to protect against such sensitization.

*b. Human studies.* The metabolism of MC to CO and measurement of COHb in human subjects exposed to MC were described in detail in the NPRM. In summary, it was found that exercising increased MC uptake and, subsequently, increased blood COHb levels compared to that of sedentary individuals [Ex. 7-222]. In addition, COHb levels due to smoking were found to be additive to the COHb produced by MC metabolism. Taken together, these results suggested that smokers or individuals engaged in physical exertion (as in a workplace) may be at increased risk from CO-induced toxicity from MC exposure. This risk may be especially elevated in individuals with silent or symptomatic cardiac disease who may be susceptible to very small increases in COHb because of an already impaired blood supply to the heart. Many American workers have silent or symptomatic heart disease. This increased OSHA's concern for the potential cardiac effects of MC and its metabolites.

Elevated COHb has been measured in humans experimentally and occupationally exposed to MC [Exs. 7-4, 7-5-R0327, 7-102, 7-115, 7-157, 7-159, 7-169, 7-174, 7-176]. The effects of elevated COHb are primarily increased risk of myocardial infarction, especially in susceptible individuals. Atkins and Baker [Ex. 7-198] described two cases of myocardial infarction in workers subsequent to CO exposure. COHb was measured at 30% and 24% in these individuals, which is much higher than normal general population levels of COHb. Humans exposed to MC would not be expected to experience COHb at those levels unless the exposure to MC was extremely high (greater than 500 ppm).

In a laboratory study of humans with coronary artery disease, subjects were exposed to CO and observed for cardiac health effects during exercise. In subjects with 3 to 10% COHb, decreased exercise tolerance and increased anginal pain were observed [Ex. 7-198]. In an epidemiological study submitted to OSHA by NIOSH during the MC public hearings, the investigators observed a statistically significant excess of ischemic heart disease mortality among tunnel workers when compared with rates for the New York City population [Ex. 23-18]. This increase in mortality is supported by clinical findings. Allred et

al. [Ex. 23-18] observed that elevation of COHb from 0.6% to as low as 2% decreased time to myocardial ischemia and anginal pain during laboratory tests. OSHA believes that these studies, taken together, suggest that small increases in COHb can adversely affect persons with compromised cardiac health. The results observed in the tunnel workers are particularly relevant because they show an increased risk in a working population. NIOSH used these studies to support its recommendation that the COHb effects of MC be carefully considered in the MC rulemaking [Tr. 881-2, 9/21/92]. OSHA agreed with NIOSH that the effects observed at low levels of COHb are cause for concern about the risks of MC metabolism to CO.

In the NPRM, OSHA also reviewed case reports in which individuals exposed to MC experienced myocardial infarctions [Exs. 7-102, 7-73]. These case reports suggested that exposure to MC increased cardiac stress, although it was not determined whether this was a direct effect of MC or as the result of metabolism of MC to CO. OSHA believes that these case studies support the hypothesis that CO generated through metabolism of MC would have the same adverse health effects as direct CO exposure.

Two epidemiological studies (in film coating and fiber production workers) [Exs. 7-75, 7-76, 7-122, 7-163] examined cardiac mortality due to occupational exposure to MC. Ott [Ex. 7-76] compared mortality from a plant in South Carolina that used MC to a reference plant in Virginia. An increased risk ratio for ischemic heart disease (risk ratio = 3.1) was observed in the MC-exposed workers compared to the reference population.

This approach controls for the healthy worker effect by comparing two working populations, and excess risk was demonstrated. The authors believed that the apparent excess risk was due to geographical variability in the incidence of ischemic heart disease. The population from the reference plant was found to have an unusually low death rate due to ischemic heart disease in comparison to the general population rate.

In an update of the study [Ex. 7-75], the ischemic heart disease rate in the exposed population was compared to that in the surrounding York County, S.C. population instead of a reference plant. No difference in ischemic heart disease rates was detected between exposed workers and controls, although this approach would not control for the healthy worker effect. The SMR was 0.94 (32 observed, 34.2 expected).

NIOSH disagreed with the conclusion of the authors of this study, and indicated that the studies summarized above would be cause for concern regarding the cardiac effects of MC. NIOSH suggested that the raw data from the epidemiological studies of cellulose acetate film production workers and the studies of workers in cellulose acetate fiber manufacture be reviewed for cardiac mortality occurring during the period of occupational exposure for the workers. OSHA is concerned about the potential CO effects from metabolism of MC and will continue to monitor the scientific literature on this topic. However, the Agency is setting the exposure limits based on cancer and CNS effects and has not reached final conclusions on this issue.

### 3. Hepatic Toxicity

Chlorinated hydrocarbons as a class, such as carbon tetrachloride and chloroform, are toxic to the liver. In general, chlorinated hydrocarbons cause cytotoxicity (cell death) in rodent livers. Therefore, there was suspicion that the liver would also be a target organ for MC (a chlorinated hydrocarbon) toxicity. OSHA evaluated the available literature on the hepatic effects of MC in animal and human studies.

*a. Animal studies.* Studies of the effects of MC exposure on the rodent liver have not demonstrated significant acute liver toxicity, even at lethal or near-lethal doses. As summarized in the NPRM, Kutob et al. [Ex. 7-27] and Klaassen et al. [Ex. 7-28] conducted experiments on halogenated methanes and hepatotoxicity. MC was determined to be the least hepatotoxic of the halogenated methanes examined. The only injury described was a mild inflammatory response associated with lethal MC concentrations. These studies demonstrated that liver was not the primary target organ for the acute toxicity of MC.

Weinstein et al. [Ex. 7-181] examined the hepatic effects of MC on female mice who were continuously exposed for up to 7 days to MC concentrations of up to 5000 ppm. Mild, nonlethal injury to the livers was noted, characterized by balloon degeneration of the rough endoplasmic reticulum (RER), transient severe triglyceride accumulation (fatty liver), partial inhibition of protein synthesis and breakdown of polysomes into individual ribosomes. The injury is similar to a mild form of carbon tetrachloride toxicity (a structural analog of MC) and suggests that although the toxicity due to MC is not as severe as that produced by carbon tetrachloride, the mechanism of toxicity may be similar.

In subchronic experiments more severe effects were observed in the liver after continuous exposure. MacEwen et al. [Ex. 7-14] studied the effects of continuous exposure of mice, rats, dogs and rhesus monkeys to 1000 and 5000 ppm MC for up to 14 weeks. Fatty liver, icterus, elevated SGPT and ICDH were reported in dogs at both concentrations. These effects appeared at 6-7 weeks of exposure to 1000 ppm MC and at 3 weeks of exposure to 5000 ppm. Monkeys were less sensitive to hepatic injury, and showed no changes in liver enzymes and only mild to moderate liver changes at 5000 ppm MC. No liver alterations were detectable in monkeys exposed to 1000 ppm MC. Mice and rats developed liver toxicity at both exposure levels, characterized by increased hemosiderin pigment, cytoplasmic vacuolization, nuclear degeneration and changes in cellular organization.

Hepatic effects associated with chronic MC exposure were observed in lifetime cancer bioassays in three rodent species: rats, mice and hamsters. In studies conducted by the NTP and Dow Chemical Co., rats were exposed to inhalation concentrations of MC from 50 ppm to 4000 ppm 6 hours per day, 5 days per week [Exs. 7-8, 7-151, 7-173]. Hepatic effects were observed after exposure to MC concentrations as low as 500 ppm. These effects were characterized by increased fatty liver, cytoplasmic vacuolization and an increased number of multinucleated hepatocytes. At higher doses (greater than 1500 ppm), increased numbers of altered foci and hepatocellular necrosis became apparent.

Serota et al. [Ex. 7-180] administered 5 to 250 mg MC/kg body weight to rats in drinking water. Hepatic toxicity similar to that observed in the inhalation studies was reported at doses from 50 to 250 mg/kg.

In mice, the chronic hepatic effects of MC were investigated in two bioassays: NTP [Ex. 7-8] and Serota et al. [Ex. 7-179]. In the NTP study, mice were exposed by inhalation to 2000 or 4000 ppm MC. Cytologic degeneration was observed in both male and female mice and increased incidences of hepatocellular adenomas and carcinomas were found at both concentrations. The carcinogenic effects of MC are described in greater detail above, in the discussion of MC carcinogenicity.

In a drinking water study, Serota et al. found that mice exposed to 50 to 250 mg/kg/d MC had dose-related increases in the fat content of the liver (a sign of liver toxicity). Although some proliferative hepatocellular lesions were

identified in this study, they were distributed across all exposure groups. Hepatocellular tumor incidences were not elevated above historical control incidences.

In the hamster, Burek et al. [Ex. 7-151] found minimal treatment-related changes in the livers of the MC-exposed animals after exposure to 500, 1500 or 3500 ppm MC. A dose-related increase in hemosiderin was found in male hamsters at 6 months and at 3500 ppm at 12 months. No other changes in liver physiology were reported.

OSHA believes that these studies demonstrate that the rodent liver is not sensitive to acute effects of MC, but that chronic exposure to MC caused toxic effects in rat and mouse liver and cancer in mouse liver. These studies appear to have been well conducted and the differences in toxicity observed across studies were likely due to differences in dose or route of exposure. The hamsters appeared to be insensitive to liver toxicity. OSHA believes that this is most likely due to inherent species differences in response to toxicants.

*b. Human studies.* OSHA evaluated epidemiological studies and case reports to determine the extent of hepatic effects detected after exposure of humans to MC. Liver toxicity was measured as alterations in the blood levels of any of several normal liver enzymes in these studies.

*i. Epidemiological studies.* In a cross-sectional analysis of the health of workers in an acetate fiber production plant in which workers were exposed to 140 to 475 ppm MC, Ott et al. [Ex. 4-33c] reported statistically significant increases in serum bilirubin and alanine aminotransferase (ALT) (also known as serum glutamic pyruvic transaminase (SGPT)) when compared with a reference group of industrial workers. The elevation in bilirubin levels showed a dose-response relationship, but the ALT levels were not associated with MC exposure. The authors felt that the increase in ALT in MC-exposed workers could not be attributed to MC because a dose-response relationship was not demonstrated and, therefore, the increase in ALT between the exposed and reference populations could be disregarded as a sign of liver toxicity. The authors concluded that although bilirubin elevation may be interpreted as a sign of liver toxicity, this interpretation was not supported by alterations in other liver parameters. OSHA feels that ALT cannot be disregarded as unrelated to MC exposure based on the lack of dose response within the exposure group. The high variability of this parameter and the low numbers of individuals

within certain exposure subgroups (e.g., 10 men exposed at 280 ppm), make a dose-response relationship more difficult to demonstrate. Any mistake made in the characterization in an exposure group would result in obscuring the dose-response relationship. Although the evidence is not unequivocal, OSHA believes that the elevated bilirubin coupled with the elevated ALT values indicate suggestive evidence of a hepatotoxic response to MC exposure in this worker population.

In an update to the study described above, Cohen et al. [Ex. 7-75] found 4 cases of liver/biliary duct cancer in workers with more than 10 years of exposure to MC and after 20 years from first hire. Further description of this study can be found in the discussion of MC carcinogenicity, above.

In an English translation of a 1968 Czechoslovakian study, Kuzelova et al. [Ex. 7-26] found no liver enzyme abnormalities in workers exposed to MC concentrations from 29 ppm to 4899 ppm for up to two years. In contrast, in an English translation of a German study which focussed on neurological changes due to MC exposure, Hanke et al. [Ex. 7-195] observed pathological liver function tests and hepatomegaly (enlarged liver) in 4 of 14 floor tile setters examined. These workers were chronically exposed to MC at concentrations as high as 400 to 5300 ppm. The average tenure of employment of these workers was 7.7 years. The authors of the Hanke study noted that although MC with its impurities could be responsible for the liver damage, the evidence was not conclusive. OSHA has determined that there is insufficient evidence from the Kuzelova and Hanke studies to conclude that MC causes chronic human hepatotoxic effects.

*ii. Case reports.* In addition to the cross-sectional analyses of worker morbidity described above [Exs. 4-33c and 7-26], the relationship of MC exposure and hepatotoxicity has been studied by analysis of case reports. Welch [Ex. 7-73] collected 144 case reports of clinical disease reported subsequent to occupational MC exposure. Quantitative exposure estimates for individuals were unreliable, but the presence of MC in the work environment was ascertained for each employee. The most prevalent findings in these case reports were CNS symptoms, upper respiratory syndrome and alterations in liver enzymes. The patterns of alteration in liver enzymes were not consistent among individuals, but may be suggestive of a MC-associated hepatotoxic effect. One case of hepatitis of unknown etiology was identified. The case physician believed

that the hepatitis was secondary to solvent exposure. The solvents to which this employee was exposed included xylene and methylethyl ketone as well as MC. OSHA believes that the confounding solvent exposures in the hepatitis case and the unknown exposure histories of the individuals with altered liver enzymes limit the interpretation of these studies. OSHA has determined that these case reports provide insufficient evidence to conclude that MC was the causative agent in these cases.

Analysis of cases of fatal and near-fatal human exposures [Exs. 7-18, 7-19] indicated no apparent acute alterations of liver function. Acute concentrations of MC which caused narcosis and even death were not associated with changes in liver enzymes.

OSHA concludes that limited evidence supports the hypothesis that MC causes human hepatotoxicity, based on the data in the Ott study. The remaining studies and case reports do not provide clear evidence of a causative role of MC in hepatotoxicity. The Agency has set the exposure limits based on cancer and CNS effects and has not reached final conclusions on this issue.

#### 4. Reproductive Toxicity

There are only limited data available regarding the potential adverse teratogenic or reproductive effects due to MC exposure. Teratogenicity studies have been conducted in rats and mice and limited epidemiology and case reports have been described for humans.

*a. Animal studies.* A study [Ex. 4-5] using chicken embryos indicated that MC disrupts embryogenesis in a dose-related manner. Since the application of MC to the air space of chicken embryos is not comparable to MC administration to animals with a placenta, the exposure effect seen in the chick embryos can only be considered as suggestive evidence that an effect may also occur in mammalian systems.

The teratogenicity of inhaled MC has also been studied in rats and mice [Exs. 7-20, 7-21, 7-22]. In 1975, Schwetz et al. [Ex. 7-21] conducted a study on Swiss Webster mice. Mice were exposed to 1250 ppm MC for 7 hours/day, on days 6-15 of gestation. On day 18 of gestation, Caesarian sectioning of dams was performed. A statistically significant increase in mean maternal body weight (11-15%) was observed in dams exposed to 1250 ppm MC; however, food consumption was not measured. The only effect on fetal development associated with MC exposure was a statistically significant increase in the number of fetuses which

contained a single extra center of ossification in the sternum. The incidence of gross anomalies observed in the MC-exposed fetuses was not significantly different from that in the control litters. Maternal COHb level during exposure reached 12.6%; however, 24 hours after the last exposure, COHb had returned to control levels.

In the same study by Schwetz et al. [Ex. 7-21], Sprague-Dawley rats were exposed to 1250 ppm MC via inhalation for 7 hours daily on days 6-15 of gestation. No MC-associated effects were observed in food consumption or maternal body weight. Among litters from MC-exposed dams, the incidence of lumbar ribs or spurs was significantly decreased when compared to controls, while the incidence of delayed ossification of sternebrae was significantly increased compared to controls. No increased incidence of gross anomalies were observed in the fetuses from exposed rats compared to fetuses from control litters. No MC-associated effects were observed on the average number of implantation sites per litter, litter size, the incidence of fetal resorptions, fetal sex ratios or fetal body measurements, in the 19 litters that were evaluated. As observed in the MC-exposed mice, there was significant elevation of the COHb level in the dams, but the level returned to control values within 24 hours of cessation of exposure.

In 1980, Hardin and Manson [Ex. 7-22] evaluated the effect of MC exposure in Long-Evans rats after inhalation of 4500 ppm for 6 hours/day, 7 days/week prior to and during gestation. Four exposure groups were described. The first group was exposed to MC for 12 to 14 days prior to gestation and during the first 17 days of pregnancy. The second group was exposed to MC only during the 12 to 14 days prior to gestation. The third group was exposed to MC only during the first 17 days of pregnancy. The fourth group (control group) was exposed only to filtered air. The purpose of this study was to test whether MC exposure prior to and/or during gestation was more detrimental to reproductive outcome in female rats than exposure during gestation alone.

In rats exposed to MC during gestation, there were signs of maternal toxicity, characterized by a statistically significant increase in maternal liver weights. The only fetal MC effects observed were statistically significant decreases in mean fetal body weights. No significantly increased incidence of skeletal or soft tissue anomalies was observed in the offspring.

In 1980, Bornschein et al. [Ex. 7-224] tested some of the offspring of the Long-Evans rats from Hardin and Manson's study described above. All four treatment groups were used to assess the postnatal toxicity of MC exposure at 4500 ppm. The general activity measurements of groups of 5-day old pups showed no exposure-related effects. At 10-days of age, however, significant MC-associated effects were observed in both sexes in the general activity test. These effects were still apparent in male rats at 150-days of age. This study showed that maternal exposure to MC prior to and/or during pregnancy altered the manner in which the offspring react and adapt to novel test environments at up to 150-days of age. These effects suggest that MC exposure prior to, or during pregnancy may influence the processes of orientation, reactivity, and/or behavioral habituation. No changes in growth rate, long-term food and water consumption, wheel running activity or avoidance learning were reported.

OSHA concluded from the animal studies that maternal exposure to high concentrations of MC during pregnancy may have some adverse effects on the offspring, in particular with regard to behavioral effects. The Agency has set the exposure limits based on cancer and CNS effects and has not reached final conclusions on this issue.

*b. Human studies.* Limited data have been collected on the reproductive effects of MC in male workers. In a study reported in the Occupational Safety and Health Reporter [Ex. 7-43], a greater risk of male sterility was found in male workers exposed to MC. In 1988, Kelly [Ex. 7-165] reported 4 cases of oligospermia in MC-exposed workers. This study was described in detail in the NPRM. Although the study provided some evidence of an effect of MC on male fertility, the observations were based on a small number of cases and OSHA believes that more research is necessary before causative conclusions can be drawn about the human male reproductive toxicity of MC.

The reproductive and developmental effects due to MC exposure in female workers have also been studied. According to information reported in an English translation of an abstract of a Russian article by Vozovaya et al. [Ex. 7-16], detectable levels of MC were found in the blood, milk, embryonal, fetal and placental tissues of nursing women exposed to MC in a rubber product plant. No other information was provided in the abstract. In a study by Taskinen et al. [Ex. 7-199], increased rates of spontaneous abortions were observed in female pharmaceutical



workers exposed to MC. Exposure data were not reported in this study and it is unclear what confounding factors or other chemical exposures were present. OSHA believes that more research is necessary in order to evaluate the potential effect of MC on pregnancy outcomes, and so has not reached a conclusion on this issue.

Carbon monoxide has well known adverse reproductive effects in humans. Since MC is metabolized to CO, OSHA was concerned about the adverse reproductive effects of CO as a metabolite of MC. The EPA has reviewed the literature on the effects of maternal CO exposure on the development of the fetus in the Air Quality Criteria for Carbon Monoxide [Ex. 7-201]. Very high maternal CO exposures have resulted in fetal or infant death or severe neurological impairment of the offspring. CO reduces the amount of oxygen available to the tissues. The developing fetus is very sensitive to these effects. According to Fechter et al. [Ex. 7-200], low levels of CO exposure in animals have been shown to adversely affect the fetus, producing CNS damage or reduced fetal growth. These effects suggest that the fetus may be especially sensitive to the toxic effects of MC through its metabolism to CO.

As described above, OSHA is sufficiently concerned about the potential for reproductive health effects of carbon monoxide as a result of MC metabolism that it has decided to continue to gather information and revisit this issue, if warranted.

#### *F. Conclusion*

OSHA's determination that MC is a potential occupational carcinogen was based primarily on the positive findings of chronic inhalation bioassays in rodents. MC is carcinogenic to mice of both sexes, producing lung and liver neoplasms. In rats, MC produced dose-related increases in mammary tumors and increases in the number of tumors per tumor-bearing rat. The evidence in rodents is supported by epidemiologic findings from cellulose triacetate fiber production workers and a case-control study of individuals with astrocytic brain cancer. The study of fiber production workers suggests an association between liver and biliary cancer and long term (greater than 10 years) exposure to MC. The case-control study indicates an association between risk of astrocytic brain cancer and occupational exposure to MC. This evidence is further supported by the findings of genotoxic activity of MC in bacterial and mammalian cell systems. OSHA has set the 8-hour TWA PEL of

25 ppm primarily to protect employees from the risk of cancer due to MC exposure in the workplace.

CNS depression has been demonstrated in humans and animals at relatively low inhalation concentrations of MC. The CNS depression observed in those studies was relatively mild, although the effects occurred at concentrations in the range of the STEL of 125 ppm. OSHA believes that the STEL will be protective against CNS depression for most employees exposed to MC most of the time, but the Agency is sufficiently concerned about the potential for CNS health effects at concentrations below the STEL and have decided to continue to gather information and revisit this issue, if warranted.

#### *VI. Quantitative Risk Assessment*

##### *Summary*

After examining all the available data, both animal and human, and both quantitative and qualitative, OSHA has concluded that MC is a multi-species, multi-site carcinogen in various rodent species, and is likely to be so in humans, and that it most probably acts via one or more genotoxic metabolite(s). The evidence for this conclusion is quite strong: there exist several positive bioassays with low background incidence and dose-related increases; there is an unusually large amount of mechanistic information; and there are several positive epidemiological studies and no negative epidemiological studies of sufficient power to rule out the animal-based potency estimates.

Furthermore, OSHA has conducted a quantitative risk assessment based on the highest-quality animal tumor data, constructing a state-of-the-art physiologically-based pharmacokinetic (PBPK) model incorporating rodent and human metabolic information. That analysis shows a final estimate of risk of 3.62 deaths per 1000 workers occupationally exposed to 25 ppm MC for a working lifetime. [An alternative analysis, which incorporated all of the data used in the main analysis plus the assumption that human enzymes are even less active to MC (as compared to mice) than that predicted by the main analysis, gave a risk estimate of 1.23 deaths per 1000]. Both estimates are clearly well above any plausible upper boundary of the "significant risk" range defined by the Supreme Court, used by OSHA in its prior rulemakings, and reported in the scientific/economic literature on risk. The estimated risk at the current PEL of 500 ppm is 126 excess cancers per 1000 workers; clearly, the 25 ppm standard will effect

a substantial reduction in a very high risk. The Final Economic Analysis shows that the average risk at current exposure levels is approximately 7.6 deaths per 1000 and ranges up to 126 per 1000; at post-regulatory exposure levels (which account for the fact that the action level will encourage some employers, where feasible, to lower exposures below 25 ppm), average risk is estimated to be 1.7 deaths per 1000 (and nowhere higher than 3.62 per 1000 risk at the new PEL of 25 ppm)—also a substantial reduction of a highly significant risk.

Prior to the October 1995 record reopening, there was strong evidence to support the determination that MC is a human carcinogen, using well-established risk assessment models based on substantial biologically-based evidence and theories: there were two multi-site positive bioassays with dose-response trends and low background, and suggestive epidemiology with no clearly conflicting epidemiology. The only question was whether to use an administered-dose scaling or a PBPK model.

Data submitted in the reopening of the record in late 1995 shed light both on the hazard identification and the quantitative risk assessment. Studies of isoenzyme activity and intracellular distribution across species were interpreted by the Halogenated Solvents Industry Alliance (HSIA) to suggest that MC is not a human carcinogen. OSHA has concluded that the HSIA interpretation of the studies is not supported by the evidence. There are numerous methodological problems with the studies: for example, in the experiment in which Graves et al. examined MC-induced mutations [Ex. 123], OSHA agrees with Dr. Douglas Bell [Ex. 126-26] that insufficient numbers of doses and mutants were examined to reach any conclusions whatsoever regarding differences in mutation spectra between chemicals.

More importantly, OSHA and most commenters agreed that the data showed a quantitative—and quantifiable—difference between mice and humans, not an infinite, qualitative one. In other words, there is substantial evidence that humans and mice metabolize MC similarly, only at different rates. HSIA's qualitative argument rests on two questionable assumptions, both of which are contradicted by other data: first, that the DNA single strand break assay is infinitely sensitive—but the investigators do not even know if it is sensitive enough to show the 7-fold difference in enzyme activity between mice and humans that OSHA's main



PBPK analysis uses; and second, that the human isoenzyme most active against MC, although clearly present in human cells, is located in a different part of the cell. This interpretation: 1) contradicts some basic beliefs of comparative physiology (Why would the cell structures of humans and mice be so fundamentally different?); 2) would require OSHA to do a "subcellular PBPK analysis" to predict risk—no one has ever developed, let alone parameterized and validated, such a model; and 3) contradicts other data on activation by mouse cytosolic preparations—MC has been shown to have enhanced mutagenicity in bacterial and mammalian cell preparations when mouse cytosolic preparations were used to metabolize the MC. This requires metabolism by cytoplasmic (not nuclear) GST and for the metabolites to be stable enough to cross membranes and interact with DNA.

Therefore, the new studies do not cast doubt on the MC hazard identification—in fact, they should probably increase the level of concern because it is now more clear that MC is likely to act by a genotoxic mechanism [animal tests are most relevant to humans when clear genotoxic agents are involved] and that that pathway exists in humans, and may be concentrated in cells of concern in human cancers, such as the bile duct epithelium. OSHA notes that an epidemiologic study of cellulose triacetate fiber workers has shown a statistically significant increase in biliary duct tumors [Ex. 7-260].

On the other hand, the new data did reinforce OSHA's decision to proceed with a PBPK-based risk assessment and helped OSHA to incorporate the best available scientific data into a PBPK model. Here OSHA presents two PBPK-based risk analyses, both of which represent substantial refinements over the applied-dose risk assessment and over previous PBPK analyses. OSHA's final risk assessment incorporates all reliable data—OSHA's alternative analysis, in addition to the data in the final risk assessment, also incorporates some suggestive/sparse data found in new studies. As stated above, both analyses estimate risks at 25 ppm well in excess of any possible boundary line between significant and insignificant risk.

Both of OSHA's PBPK analyses made two major advances: 1) the use of non-independent Monte Carlo simulation—Monte Carlo simulation is a well-developed computational technique that allows the modeler to take estimates of uncertainty in each of the many variables in a complex model and generate a quantitative estimate of the

total uncertainty in the result. Others have used Monte Carlo simulation in PBPK modeling, but OSHA added information on the covariance structure of all the parameters, so that the uncertainty estimate would not be biased (exaggerated, probably) by incorrectly assuming that all the variables could simultaneously be at their lowest or highest values; and 2) the use of Bayesian analysis—this allows uncertainty distributions to be better estimated (narrowed) by cross-checking them against other independently-collected data from laboratory experiments, rather than simply guessing how big the uncertainties are and not refining the estimates as the model runs.

Both these advances enabled OSHA to strike a balance between two unsatisfactory extremes—a) the extreme overconfidence of using estimates for each variable that did not allow for any uncertainty—or b) the extreme "underconfidence" of assuming that all uncertainties are independent of each other and of other laboratory data. The result is an analysis that tells what science knows and does not know about the relationship between ambient concentrations and the putative relevant dose measure (concentration of GST metabolites in the target organ) in mice and humans.

Again, OSHA's final risk assessment regards the very limited human data base on GST-0 activity [a total of 39 liver samples and 5 lung samples] as useful, but insufficient to discard the traditional "allometric" assumption (the well-validated assumption that, as a general rule, metabolic parameters scale proportional to a function of the animal's body weight). OSHA's alternative analysis accepts the limited human data at face value to extrapolate without using allometry. OSHA has concluded that the main analysis is better supported by available evidence than is the alternative analysis, but both yield significant risks. An important caveat is that both models are strictly applicable to humans who are physiologically similar to the six subjects analyzed by Dow (see the discussion later in this document for a fuller explanation). Since the population of 200,000 workers will be much more heterogeneous than those six subjects, we regard these estimates as "overconfident"—some workers exposed at 25 ppm will have higher risks than 3.6 per 1000 (although some may have lower risks as well).

#### Introduction

OSHA performs quantitative risk assessment, when information permits,

to help determine the Permissible Exposure Limit (PEL) for toxic substances (contingent on the feasibility determination). The first step of assessing risks to human health is hazard identification. This step results in the determination that an exposure to a toxic substance causes, is likely to cause, or is unlikely or unable to cause, one or more specific adverse health effect(s) in workers. This identification also shows which studies have data that would allow a quantitative estimation of risk.

If studies are available that contain information regarding the amount of exposure and disease, mathematical modeling allows extrapolation of the information in the study to conditions of concern in the workplace. OSHA uses these risk estimates to determine whether exposure results in significant risk, and whether the standards considered by OSHA substantially reduce the risk.

This section describes the record evidence received during the public rulemaking concerning OSHA's quantitative risk assessment and the reasons OSHA has maintained or modified its opinion from the proposal. In the following sections, the evidence supporting and casting doubt on the hypothesis that MC is a probable carcinogen (the "Hazard Identification" issues) is discussed first. Then the results of OSHA's quantitative risk assessments, conducted to estimate the carcinogenic potency of MC, are discussed.

#### A. Methylene Chloride Hazard Identification

Animal and human evidence, summarized in the health effects section, indicates that MC can cause cancer, cardiac effects, central nervous system damage and other health effects. As described in the NPRM, OSHA's preliminary quantitative risk assessment was based on cancer and relied on rodent bioassay data for quantitation of risks. In 1986, the National Toxicology Program (NTP) concluded that the mouse bioassay data provided "clear evidence" of carcinogenesis in male and female mice, based on the liver and lung tumors. The NTP also determined that the rat mammary tumors observed in the bioassay provided clear evidence of carcinogenesis in female rats and some evidence of carcinogenesis in male rats. This evidence of cancer in multiple species and in both sexes underlies the concern for MC as a potential human carcinogen. On the basis of these studies, IARC has classified MC as a 2B carcinogen, the EPA has classified MC as a B2 carcinogen and NIOSH has

classified MC as a potential occupational carcinogen. OSHA concurred with these assessments.

Animal bioassays are a critical tool in determining the potential hazard of a substance for humans. Virtually all of the toxic substances that have been demonstrated to be carcinogenic in humans are also carcinogenic in laboratory animals. Although it is possible that a substance may be carcinogenic in a laboratory species, but not in humans, it is reasonable to suspect that substances that cause cancer in multiple animal species and at multiple target organ sites would be carcinogenic in humans. Therefore, in the absence of sufficiently powerful negative epidemiological studies or mechanistic studies demonstrating that the purported carcinogenic mechanism of action of the substance is irrelevant to humans, OSHA and other federal agencies rely on well-conducted, high-quality bioassays as the primary basis for their hazard identification and risk assessment. This is the case with MC.

During this rulemaking, some commenters have supported and others have questioned the hazard identification of MC as a potential human carcinogen. Most recently, some commenters contested the relevance of the mouse bioassay data for extrapolating to human cancer risks. Although these issues were raised by some rulemaking participants earlier in the rulemaking process, they were most thoroughly explored in connection with the information received by the Agency in late 1995. On October 24, 1995, OSHA reopened the MC record to receive comments on several studies submitted to the Agency by the Halogenated Solvents Industry Alliance (HSIA) pertaining to the mechanism of action of MC carcinogenesis in mice, and the implications of these studies for estimating human risks. The record closed on November 29, 1995, but was reopened in order to give the public additional opportunity to comment on the submitted studies. The record then closed on December 29, 1995. Thirty-seven comments were received on this topic and reviewed as part of this rulemaking.

The papers submitted by the HSIA consisted of a cover letter [Ex. 117], an overview of the sponsored research [Ex. 118] and seven research papers on the mechanism of MC carcinogenesis [Ex. 119-124A]. The hypothesis under investigation in these seven studies was that the pathways of MC metabolism and the mechanism of carcinogenesis in the mouse represented a unique situation that would not take place in humans, making the mouse unsuitable

as the basis for extrapolating risks of cancer to humans. The specific studies are described briefly here and the comments received during the reopening of the rulemaking record are discussed in detail below.

#### 1. Summary of Studies Submitted by HSIA

*Exhibit 119 "Methylene Chloride: an inhalation study to investigate toxicity in the mouse lung using morphological, biochemical and Clara cell culture techniques," J.R. Foster, T. Green, L.L. Smith, S. Tittensor, and I. Wyatt, Toxicology 91 (1994) 221-234.*

This study investigated the potential role of MC as a mouse lung carcinogen via non-genotoxic mechanisms and the Clara cell as the cell of origin in mouse lung cancer. The hypothesis was that MC acts specifically to produce toxicity (vacuolation) in Clara cells which leads to cell proliferation and production of mouse lung tumors. The authors investigated the toxicity of MC in bronchiolar Clara cells by measuring the production of vacuoles after exposure to MC. The investigators also measured DNA synthesis in Clara cells isolated from mice exposed to MC as a measure of cell proliferation.

The authors observed a transient vacuolation of bronchiolar Clara cells in mice exposed to 2000 and 4000 ppm MC, but not in mice exposed to 0, 125, 250, 500 or 1000 ppm MC. When the mixed function oxidase (MFO) pathway was inhibited, the bronchiolar cell vacuolation observed after exposure to 2000 and 4000 ppm MC was reduced. Inhibition of the glutathione S-transferase pathway (GST) had no effect on Clara cell vacuolation. The researchers also found that exposure of mice to 1000 ppm MC or greater for 6 hours induced an increase in DNA synthesis in Clara cells cultured *in vitro* from exposed animals.

Clara cells are present in mice, rats and humans, but appear to be more abundant in mice than other species. Clara cells contain enzymes for both the MFO and glutathione S-transferase (GST) pathways of MC metabolism. According to the authors, the results of this study suggest that metabolism of MC via the MFO pathway induces a transient toxicity in Clara cells and a transient increase in DNA synthesis.

*Exhibit 120 "Methylene chloride-induced DNA damage: an interspecies comparison," R.J. Graves, C. Coutts and T. Green, Carcinogenesis, vol. 16 no. 8 pp. 1919-1926, 1995.*

This study investigated the role of MC as a mouse carcinogen via a genotoxic mechanism of action. The hypothesis under investigation was that MC is

metabolized to a genotoxic carcinogen via the GST pathway to different extents in different species and that expression of this genotoxicity correlates with risk of developing cancer across species. The authors used production of single strand (ss) DNA breaks as a measure of genotoxicity. The researchers measured DNA ss breaks in lung and liver cells from mouse, rat, hamster and humans. They observed increased DNA ss breaks in mouse liver cells, after *in vivo* exposure to 4000-8000 ppm MC for 6 hr and in mouse lung cells after exposure to 2000-6000 ppm MC. Depletion of glutathione in the liver (after administration of buthionine sulfoximine) reduced the amount of ss breaks observed. No increase in ss breaks was observed in Clara cells isolated from mice exposed to MC *in vivo*. However, in experiments on isolated mouse Clara cells, the authors observed increased DNA ss breaks in cells exposed to concentrations of MC of 5 mM and above.

No increases in ss breaks above control levels were detected in rat livers after exposure to 4000 ppm for 6 hr or in rat lungs after exposure to 4000 ppm for 3 hr. Increases in ss breaks were also not detected in hamster and human liver cells after exposure to MC *in vitro* at concentrations up to 90 and 120 mM.

In Chinese hamster ovary (CHO) cells, MC plus mouse liver cytosol (which contains the GST enzymes) also induced ss breaks, while incubation of CHO cells with MC in the presence of mouse liver microsomes (which contain the MFO enzymes) did not increase ss breaks.

The results suggest that mouse liver and lung cells are more susceptible to MC-induced ss breaks than cells from rats, hamsters or humans. Assuming that ss breaks are a relevant surrogate for carcinogenicity, the authors infer from this study that humans, rats and hamsters are insensitive to MC-induced liver cancer, because those species lack the high level of GST metabolic activity to MC found in the mouse liver cell and lung Clara cell.

*Exhibit 121 "Isolation of two mouse theta glutathione S-transferases active with methylene chloride," G.W. Mainwaring, J. Nash and T. Green, Zeneca Central Toxicology Laboratory, 1995.*

This study was conducted in order to characterize the mouse GST isozyme(s) responsible for MC metabolism. The results of this work could be used to explore the hypothesis that a particular GST isozyme was responsible for metabolizing MC to the carcinogenic metabolite and that there may be different concentrations of this enzyme across species.

The researchers used a variety of chromatography methods to isolate two mouse glutathione S-transferases (MT-1 and MT-2, also known as T1-1\* and T2-2\*, respectively) which metabolize MC, comparing the observed enzyme activity with that described in rats. Rats were found previously to have two GST isomers in the theta class (GST 5-5 and GST 12-12) which metabolized MC. The mouse MT-1 and MT-2 enzymes were found to be closely related to rat GST 5-5 and 12-12, respectively, and the specific activity of mouse MT-1 was found to be similar to rat GST 5-5. GST 12-12 and MT-2 were found to be extremely labile during purification, and so the specific activities of those isozymes have not been measured.

The results of this study suggest that the mouse and rat contain GST theta enzymes similar in amino acid sequence and in specific activity (GST 5-5 and MT-1). The authors postulate that the greater conjugating activity seen in mice in other studies is "probably due to a difference in expression of the enzyme or to a significant contribution from MT-2" [Ex. 121].

*Exhibit 122 "Mouse Liver glutathione S-Transferase Mediated Metabolism of Methylene Chloride to a Mutagen in the CHO/HPRT Assay," R.J. Graves and T. Green, Zeneca Central Toxicology Laboratory, 1995.*

This study investigated the mutagenicity of MC as a potential carcinogenic mechanism of action. The purposes of this study were to clarify the ability of MC to act as a mutagen, because studies in mammalian systems have yielded mixed results regarding the mutagenicity of MC, and to more fully characterize the metabolite purportedly responsible for MC mutagenicity by comparing the results to formaldehyde (one metabolite of MC by the GST pathway). Mutagenicity was measured by assaying CHO cells *in vitro* for mutations at the HPRT locus of DNA. Ss DNA breaks were also monitored. Cells were exposed in culture to MC mouse liver cytosol metabolites (which include metabolic enzymes for the GST but not the MFO pathway), formaldehyde (one of the MC GST metabolites) or 1,2-dibromoethane (1,2-DBE) (a reference genotoxin).

Using standard techniques, MC GST metabolites were shown to be weakly mutagenic using the CHO/HPRT assay. Formaldehyde was also determined to be weakly mutagenic in this assay, but the effect was not as great as with MC GST metabolites. 1,2-DBE, as expected, showed a potent mutagenic response. The mutagenicity of MC GST metabolites and formaldehyde was increased when cell density was

increased, cells were exposed in suspension rather than as attached cultures and cytosol concentration was optimized.

MC mouse liver cytosol metabolites were observed to increase ss DNA breaks in CHO cells exposed in suspension, but caused only marginal increases in DNA-protein cross-links. In contrast, the researchers found that formaldehyde induced both DNA ss breaks and DNA-protein cross-links. Slight increases in ss DNA breaks were also seen with exposure to either MC alone or the cytosol fraction alone.

Based on a comparison of the mutagenic effects of the three compounds, particularly on the lack of MC-induced DNA-protein cross-linking in this experimental system, the authors concluded that formaldehyde does not play a major role in MC mutagenicity. Accordingly, the researchers viewed the results of this study as supporting the hypothesis that the DNA ss breaks induced by MC, and the resultant DNA mutations, are caused by interaction of S-chloromethyl-glutathione (formed by the GST pathway) with DNA.

*Exhibit 123 "DNA Sequence Analysis of Methylene Chloride-Induced HPRT Mutations in CHO Cells: Comparison with the Mutation Spectrum Obtained for 1,2-Dibromethane and Formaldehyde," R.J. Graves, P. Trueman, S. Jones and T. Green, Zeneca Central Toxicology Laboratory, 1995.*

The purpose of this study was to describe the types of mutations induced by MC in order to further characterize the GST metabolite likely to cause MC mutations and therefore perhaps be responsible for the carcinogenicity of MC in the mouse. The spectrum of mutations in the HPRT locus of CHO DNA induced by MC plus mouse liver cytosol was compared to mutations induced by formaldehyde (a GST metabolite of MC) or 1,2-dibromoethane (1,2-DBE, a reference genotoxin).

The results were expressed as a sequence analysis of 11 MC-induced mutations, 6 formaldehyde-induced mutations and 13 1,2-DBE-induced mutations. In comparing the distribution of types of mutations, the results suggested to the researchers that formaldehyde-induced DNA damage can contribute to MC mutagenicity, but that the majority of the mutations were derived from other types of DNA damage, probably via an interaction of S-chloromethylglutathione with DNA. The researchers noted that a glutathione conjugate also plays a role in the mutagenicity of 1,2-DBE. The increases above background mutation frequency detected through this study were 24.7-

fold for 1,2-DBE, 4.7-fold for formaldehyde, and 8-fold for MC.

*Exhibit 124 "The distribution of glutathione S-transferase 5-5 in the lungs and livers of mice, rats and humans" [Preliminary communication, T. Green, 1995].*

*Exhibit 124A "The distribution of theta class glutathione S-transferases in the liver and lung of mouse, rat and human." G.W. Mainwaring, S.M. Williams, J.R. Foster and T. Green, 1995.*

The preliminary communication [Ex. 124] and the unpublished report which followed [Ex. 124A] summarized the results of a study comparing the inter- and intra-cellular distribution of the messenger RNA (mRNA) for a glutathione S-transferase (GST) isoenzyme which metabolizes MC in the lungs and livers of mice, rats and humans. The purpose of the experiments summarized in these reports was to describe the distribution of the mRNA for the GST theta isozyme believed to be responsible for metabolism of MC to a carcinogenic metabolite in different species. The researchers believed that differences in distribution of the mRNA for this isozyme would correlate with differences in distribution (and activity) of the isozyme itself, and might explain differences in sensitivities of the species to the carcinogenicity of MC.

The distribution of GST theta mRNA was visualized using DNA oligonucleotide anti-sense probes complementary to the nucleotide sequences for the GST theta isozymes. This technique is used to visualize the mRNA coding for a specific protein (such as the GST theta isozymes) within cells in tissues. The mRNA is a nucleotide sequence transcribed from the DNA containing the gene for the specific protein. After transcription, mRNA is transported to the cytoplasm, where it is translated into the amino acid sequence which becomes the specific protein (in this case, the GST theta isozyme). The finished protein then migrates to its final site of activity within the cell. Localization of the mRNA does not necessarily correspond to localization of the specific protein.

The results of the study showed that the GST-specific mRNA could be found in lungs and livers of all three species. Mouse liver cells (particularly the nuclei) and mouse lung cells appeared (from the photomicrographs shown in the article) to stain more heavily for the GST mRNA than the lung or liver cells from rats or humans. Although the amount of GST-specific mRNA was not quantified in this study, the authors interpreted the photographs to suggest that, "mouse tissues are stained

much more heavily than sections from either rat or human." Based on the intracellular and intercellular distribution of the GST mRNA, the authors stated,

The most significant findings are the presence of very high concentrations of GST 5-5 mRNA in specific cells and nuclei of mouse liver and lung. Metabolism of methylene chloride at high rates and within nuclei to a reactive but highly unstable glutathione conjugate is believed to facilitate alkylation of DNA by this metabolite. The lack of high or nuclear GST 5-5 concentrations in rat and human tissue, provides an explanation for the lack of genotoxicity in these species. [Ex. 124]

In the letter submitting the studies summarized above to OSHA, HSIA characterized the studies as follows:

This research, which is now complete, shows that B6C3F1 mice \* \* \* are uniquely sensitive at high exposure levels to methylene chloride-induced lung and liver cancer, and that other species, including humans, are not at similar risk. [Ex. 117]

They went on to conclude:

As a result of this research program, it appears that there are no foreseeable conditions of human exposure in which the carcinogenic effects seen in mice would be expected to occur in man. \* \* \* The risk assessment that is the basis for the methylene chloride standard, which is in turn based on the increased liver and lung tumor incidence observed in the mouse bioassay, must be discarded in favor of scientific data that are relevant to human risk.

In response to the request by HSIA, OSHA has reviewed the cancer hazard identification of MC based on all of the evidence in the MC record, with particular emphasis on the validity of the conclusion stated immediately above. This review is presented below.

## 2. Carcinogenesis of Methylene Chloride

*a. Animal evidence.* Several long-term MC bioassays have been conducted and are summarized in the Health Effects section. These included studies in which the route of exposure was inhalation [Burek et al., Ex. 4-25, Nitschke et al., Ex. 7-29, and NTP, Ex. 4-35] and two studies in which the route of exposure was drinking water [National Coffee Association, Exs. 7-30, 7-31]. In order to ensure full consideration of the data, OSHA analyzed in its preliminary assessment all data sets which showed an elevated incidence of tumors in a MC-exposed group, compared to controls, whether or not the elevation of tumor response was statistically significant. This analysis and the individual datasets used were described in detail in the NPRM.

In the NTP bioassay [Ex. 4-35], groups of 50 nine-week old B6C3F1

mice of each sex were exposed by inhalation to 0, 2000 or 4000 ppm MC. Groups of 50 eight-week old F344/N rats of each sex were exposed to MC at concentrations of 0, 1000, 2000, or 4000 ppm. The inhalation exposures were administered 6 hours a day, 5 days a week for 102 weeks. Food was provided to the animals ad libitum except during the exposure periods, while water was available at all times via an automatic watering system. All animals were observed twice a day for mortality and moribund animals were sacrificed. Clinical examinations were performed once a week for 3.5 months, then twice a month for 4.5 months, and once a month thereafter. Each animal was also weighed weekly for 12 weeks, then monthly until the conclusion of the study at 102 weeks. All animals were necropsied and histologically examined. Three different neoplastic lesions were observed to have significantly increased incidence over the controls: adenomas and carcinomas of the lung in male and female mice, adenomas and carcinomas of the liver in male and female mice, and mammary gland fibroadenomas and fibromas in male and female rats.

HSIA and others argued that benign tumors, especially the mammary tumors in the rats, should not be counted as a carcinogenic response. The NTP has addressed that issue in its Technical Report [Ex. 4-35] and has concluded that the benign mammary tumors observed in the F344 female rats are "clear evidence" of carcinogenicity and noted that such tumors may proceed to malignancy. OSHA agrees with this determination and has considered the rat mammary tumors as part of its cancer hazard identification for MC. However, OSHA's quantitative risk assessment does not consider rat mammary tumor responses.

OSHA believes that the NTP studies provide the strongest evidence of carcinogenicity of MC in animals. Many commenters and hearing participants [Exs. 19-46, 7-128, 7-126, 25-E, 126-11, 126-12, 126-16 and others] supported the use of the NTP mouse study as the basis for quantitative risk assessment. There are several reasons for this described in the proposal and earlier in this document. In brief, the NTP study used well established standard operating procedures that are generally considered a predictor of a potential carcinogenic response in humans. This study was also replicated by a second partial bioassay, conducted by NTP, in which groups of female mice were exposed to 2000 ppm MC for 2 years. Statistically significant increases in alveolar/bronchiolar and

hepatocellular tumors were observed [Ex. 27].

Before the 1995 record reopening, some commenters had raised specific arguments why a mouse study might not predict human carcinogenic response to MC. Mr. Krenson of Besway Systems [Tr. 397, 9/17/92] objected to OSHA using the NTP mouse study as the basis for setting the PELs for MC. He believed that the mouse was irrelevant to human risk because the doses used were "extremely high" and that he believed that tests conducted on rats, hamsters and human epidemiological investigations showed "no conclusive proof of cancer in human beings." OSHA disagrees with Mr. Krenson's conclusion. In general, high doses in rodent bioassay studies are appropriate to elicit a response due to the practical limitations on the number of animals that can be used in a study. In MC, there was no observed acute toxicity at the levels used in the study, which is an indication that the doses were not too high. Use of high doses in bioassay studies is common and its practical necessity has been affirmed by numerous expert bodies, including several committees of the National Academy of Sciences. In addition, for every known human carcinogen, positive results were obtained at high rodent doses. Also, quantitative comparisons, as conducted by Allen and Crump in 1988, demonstrate that, in general, observations of cancer potency from epidemiology studies agree with estimates of potency derived from rodent bioassay data. In the case of MC, statistically significant excess tumors were observed in mice after exposure to only 2000 ppm, or only four times the former PEL of 500 ppm (8-hour TWA), and excess tumors were seen in rats at 4000 ppm. This level is within the range of human exposures experienced in occupational settings. Certainly the lower exposure showing substantial effect was not "extremely high" in relation to the exposure limit, as Mr. Krenson claimed.

The HSIA and several others [Exs. 117, 126-1, 126-3, 126-5, 126-6, 126-8, 126-10, 126-13, 126-20, 126-21, 126-29] also objected to using the mouse data as the basis of human risk assessment, based on the mechanism of action studies submitted to the Agency by HSIA on December 6, 1995. OSHA's analysis of the individual studies follows, but overall, the Agency has determined that the mouse cancer data are appropriate for assessment of the cancer risks to humans (although, as discussed later in this section, OSHA has made extensive use of the submitted data to modify the quantitative

estimates of risk derived from the mouse model).

*b. Evidence pertaining to the mechanism of action of methylene chloride.* Several lines of evidence relate to the mechanism of carcinogenesis of MC. The issues discussed in the papers submitted by the HSIA and subsequent comments can be divided into those pertaining to genotoxicity, those discussing potential non-genotoxic modes of action, and those related to the enzymatic metabolism of MC. Although some comments overlap these divisions, this organization is used in this discussion to simplify consideration of the issues.

*(1) Genotoxicity.* It has not been conclusively demonstrated that MC or its metabolites act by a genotoxic mechanism in mice and rats. Substance-specific DNA adducts, which are among the strongest evidence of direct genotoxicity, have not been identified from MC exposure. However, evidence has been accumulating that MC is likely to be carcinogenic through a genotoxic mechanism of action. For example, DNA-protein cross-links have been demonstrated in mouse liver [Ex. 21–16], increases in unscheduled DNA synthesis have been demonstrated in mouse lung [Ex. 126–25] and other evidence of MC metabolite interaction with mammalian DNA (such as increases in ss DNA breaks) has been observed. It is not necessary for a substance to bind covalently with DNA in order to act via a genotoxic mechanism, although evidence of covalent binding is a strong indication of genotoxicity. In the case of MC, although the reactive metabolites are presumed to exert a genotoxic effect by binding to DNA, no MC metabolite-DNA adducts have yet been identified. However, RNA adducts have been identified after MC exposure, which supports the hypothesis that MC acts by a genotoxic mechanism. Substance-specific DNA adducts have also not been identified for some other carcinogens which are presumed to act via a genotoxic mechanism.

In addition, as discussed in the Health Effects section, MC has been found to be mutagenic in bacterial, yeast, *Drosophila* and mammalian systems; associated with chromosomal aberrations in CHO cells; and associated with sister chromatid exchanges in mammalian cell culture systems, such as CHO and V79 cells.

Investigations of the role of metabolites of the GST pathway in the bacterial mutagenicity of MC found that in glutathione-deficient strains of *Salmonella typhimurium* MC-induced mutations were reduced [Ex. L107].

Mutation rates returned to normal when bacteria were supplemented with exogenous glutathione. This study supports the hypothesis that MC may act as a genotoxic carcinogen via its GST metabolites, although a study of similar design by Dillon et al. [Ex. 21–89] did not replicate these results.

*(i) MC induced mutations.* Studies on the MC mechanism of carcinogenesis included two studies on the mutations induced by MC in the CHO/hypoxanthine phosphoribosyl transferase (HPRT) assay. In the 1995 study by Graves et al. [Ex. 122], the investigators compared mutations induced by MC with those induced by formaldehyde and 1,2-dibromoethane. The authors characterized the results of the studies as follows:

Using the CHO/HPRT assay we have shown that MC is metabolized to a mutagen by mouse liver cytosol in a reaction which is dependent upon GST and GSH. Mutagenicity was enhanced by exposing the cells at high density in suspension rather than as attached cultures, which is consistent with the critical metabolites being extremely short-lived.

The authors also observed that the MC-induced mutations were associated with an increase in DNA ss breaks. They remarked, "The results suggest that MC-induced DNA ss breaks seen in other cell types are associated with DNA damage which can lead to mutation."

In a follow-on to the CHO/HPRT study, Graves et al. [Ex. 123] conducted a sequence analysis of HPRT mutations in CHO cells, comparing the spectrum of MC-induced mutations with those induced by 1,2-dibromoethane or formaldehyde. The investigators analyzed 28 HPRT mutations: 13 from 1,2-dibromoethane experiments, 6 from formaldehyde experiments, and 11 from MC experiments. The authors characterized their results as follows,

All three compounds induced primarily point mutations, with a small number of insertions and deletions. \* \* \* The mutation sequence results for MC suggest that formaldehyde may also play a role in MC mutagenesis, although the majority of mutations arise from other types of DNA damage, probably DNA adducts formed by reaction of S-chloromethyl glutathione with DNA.

Dr. Douglas A. Bell of NIEHS [Ex. 126–26] had specific comments regarding the study on the mutation spectra [Ex. 123]. He stated,

This experiment is extremely weak scientifically and not of publication quality. It is unlikely that such a naive experiment could detect differences in spectra between the different chemicals tested. To test the hypothesis that there are chemical specific mutation spectra requires analysis of

hundreds of mutants at several different doses. This exhibit contains no useful information for risk assessment.

OSHA agrees with Dr. Bell that there are serious methodological problems with the paper. The Agency also agrees with Dr. Bell that the important information in these two studies is that MC increases the mutation frequency, showing a clear genotoxic effect.

*(ii) Single strand DNA breaks.* In a 1995 study, Graves et al. [Ex. 120] investigated the role of MC exposure in development of single strand (ss) DNA breaks in the lung and liver of mice and rats and in hamsters and human cell cultures. The authors observed a transient, dose-related increase in DNA ss breaks in mouse hepatocytes after inhalation exposure to MC. No increased amount of ss breaks was observed in rat liver cells exposed to MC as compared to control cells. The authors also reported a decrease in the amount of ss DNA breaks in liver and lung when a glutathione depletor was administered to mice immediately before MC exposure.

In mouse and rat hepatocytes incubated with MC, the authors found increases in ss breaks, but no increases in ss breaks in hamster or human hepatocytes exposed in vitro were observed. No increase in DNA damage was observed in CHO cells exposed to MC plus mouse liver microsomes, while MC plus mouse liver cytosol induced detectable ss DNA breaks.

The authors characterized their findings in the lung as follows:

Here we show that Clara cells are also sensitive to MC-induced DNA ss breaks and that the DNA-damaging metabolites are derived from the GST pathway. \* \* \* Overall, these findings support the proposal that Clara cells are the cell of origin of MC-induced mouse lung tumors.

For liver cancer, the investigators concluded:

These studies suggest that humans (and rats and hamsters) are insensitive to MC-induced liver cancer.

Commenters raised issues about the relevance and utility of ss DNA breaks in assessing the genotoxicity of MC. Dr. Karl T. Kelsey [Ex. 126–34] and Dr. Miriam Poirier [Ex. 126–37] raised concerns about the sensitivity of the DNA ss break assay for detecting genotoxic effects.

Specifically, Dr. Kelsey stated,

Reviewing the literature, considerable weight seems to fall upon the measure of DNA single strand breaks. I have serious concerns about this assay. It is well known that the assay is extraordinarily difficult to standardize and is sensitive only to very high doses of genotoxic compounds. This data,

therefore, is certainly not compelling; persuading any competent independent scientist of its relevance to humans would be difficult.

Dr. Poirier was concerned with the sensitivity of the DNA single strand break assay and the relevance of DNA ss breaks to carcinogenesis. She remarked that ss DNA breaks and mutagenicity are secondary indicators of DNA damage. She indicated that a better measure of genotoxicity would be formation of DNA adducts. Dr. Errol Zeiger [Ex. 126–28] of NIEHS agreed, stating,

If the mechanism of carcinogenicity is through an alkylating S-chloromethyl GSH complex, there should be evidence of DNA adducts *in vitro* and *in vivo*.

OSHA agrees that DNA adducts are strong evidence of genotoxicity and that ss DNA breaks and mutagenicity are not as specific or relevant as indications of a genotoxic mechanism of action. However, the Agency has determined that, even in the absence of identified MC-specific DNA adducts, the accumulated evidence suggests that MC interacts with DNA via a genotoxic mechanism of action and that the GST pathway is a plausible carcinogenic pathway.

Dr. Melnick [Ex. 126–33] stated, “\* \* \* it has not been demonstrated that the carcinogenicity of MC in mice is dependent solely on the induction of DNA single strand breaks.” Dr. Andrew G. Salmon concurred with this analysis and also raised a serious concern about the ability of the assay even to detect increases in ss breaks, regardless of their relevance:

Green’s account states that “mouse hepatocytes were \* \* \* 20-fold \* \* \* more sensitive to the effects of methylene chloride [i.e., DNA strand breaks] than rat hepatocytes \* \* \*” and no breaks were detected in hamster or human liver cells. This is translated in the discussion to an assertion that not only humans and hamsters but also rats are completely immune to the carcinogenic effect of methylene chloride. However, the data simply do not support the assertion of a categorical difference as proposed by the HSIA. This particular work also raises a number of other issues, such as whether the liver is an appropriate model tissue, and whether single-strand breaks are an appropriate indicator of the type of genetic damage produced by the putative genotoxic metabolites of methylene chloride.

OSHA agrees that the ss DNA break assay is not as sensitive as other methodologies for assessing the genotoxic potential of MC in different systems and therefore data from the ss DNA break study must be interpreted in a quantitative, not qualitative context, with allowance for uncertainty in assay sensitivity. It is also unclear whether ss

DNA breaks are the appropriate surrogate measure for carcinogenic potential. In light of the issues raised by commenters, the Agency believes that the ss DNA break data should be interpreted with caution.

(iii) *DNA-protein cross-linking.* Casanova and Heck [Ex. 21–16] observed DNA-protein crosslinks in mouse liver, but not mouse lung, after exposure to 500, 1500 and 4000 ppm. This study indicated that metabolites of MC have the ability to interact with DNA. However, the quantity of DNA-protein crosslinks did not show a strong correlation with tumor incidence, and so the DNA-protein crosslinks were not used as a dose-surrogate for MC exposure in OSHA’s risk assessment.

The Chemical Industry Institute of Toxicology (CIIT) [Ex. 126–25] submitted further evidence that MC exposure causes DNA-protein crosslinks in mouse liver but not mouse lung, hamster liver or hamster lung. These investigators also observed RNA adducts in mouse, rat and human cells after incubation with MC, but DNA-protein cross links were only observed in the mice. In addition, they submitted a pharmacokinetic model which modeled the DNA-protein cross-links as the dose surrogate for MC exposure. Finally, they made extensive comparisons of their model with the PBPK model submitted by Clewell [Ex. 96] and EPA’s risk assessment for MC. Dr. Roger McClellan summarized the conclusions they reached as follows,

The pharmacokinetic results suggest that at very low concentrations of DCM [methylene chloride], the yield of DPX [DNA-protein cross-links] is almost linearly proportional to DCM concentration \* \* \*

DPX cannot be used directly as a surrogate for the internal dose in humans, however, because human hepatocytes, unlike mouse hepatocytes, do not appear to form DPX in measurable amounts *in vitro*. \* \* \* These results suggest that the mouse may not be an appropriate animal model for human risk assessment due to its unusual susceptibility to DPX formation and to the fact that cell proliferation is a uniquely high-dose phenomenon that may occur only in this species.

OSHA believes that this work provides more evidence for the formation of genotoxic metabolites in mouse liver after MC exposure. However, OSHA is not convinced that the DNA-protein cross-linking is the appropriate dose-surrogate for pharmacokinetic modeling. One of the strengths of Reitz’s and subsequent PBPK models was that the dose surrogate used in the modeling was linearly related to tumor incidence. That is one reason that many investigators have focused on the GST pathway,

instead of the MFO pathway of metabolism as the carcinogenic pathway. As explained by Dr. Lorenz Rhomberg [Ex. 126–16],

\* \* \* if this proportionality in the case of GST is broken by a deeper analysis, the rationale for focusing only on GST must be reevaluated.

Dr. Rhomberg was referring to results presented by HSIA on the distribution of GST theta isozymes within and among cells, but the same sentiment applies here; if OSHA were to abandon PBPK modeling using GST metabolites, all of the HSIA and other studies would have to be re-evaluated and considerable more research might need to be done. Finally, in the CIIT study, RNA adducts, a more direct measure of genotoxicity than DNA ss breaks, were observed in human hepatocytes after incubation with MC. The amount of RNA adducts in human cells was only about 3-fold lower than the amount in mouse hepatocytes. It is therefore clear that human hepatocytes in this system are forming genotoxic metabolites after exposure to MC.

OSHA notes that, in mouse lung, the DNA-protein cross-links were not observed, even though a clear dose-response relationship for tumors has been established at this site. OSHA is not convinced that the explanation for carcinogenesis in mice is DNA-protein cross-links in liver. Overall, it is unclear whether the interspecies difference in DNA-protein cross-linking is related in any way to the carcinogenic mechanism of action.

OSHA concludes that there continue to be strong reasons for using the mouse data as the basis for its quantitative risk assessment because there is a clear dose-response relationship in the mouse liver and lung tumor incidence data; the mouse metabolizes MC by the same pathways as humans; PBPK models have been developed which account for inter-species differences in MC metabolism; statistical techniques have been developed to quantify the uncertainty and variability in the parameters used in the PBPK models; and there are no data that demonstrate that the mouse is an inappropriate model for assessing human cancer risks. In fact, OSHA finds further evidence in the studies described above which suggest that MC acts via a genotoxic mechanism in human cells as well as in mice and rats, which further supports OSHA’s use of the mouse tumor incidence as the basis for quantitative risk.

(iv) *Interpreting the genotoxicity studies.* Several other issues were raised regarding interpretation of the results of

these studies on the genotoxic mechanism of action of MC. NIOSH and others [Exs. 126–30, 126–11, 126–32] commented that, in general, the data presented by HSIA supported the hypothesis that the carcinogenic metabolite(s) of MC were derived from the GST pathway. They agreed with HSIA's interpretation of the data that the studies presented here helped to confirm that the mechanism of MC carcinogenesis is through one or more genotoxic metabolites of the GST pathway.

*Interpretation of short-term effects in explaining chronic mechanisms of action.*

Concerns were raised about the generalizability of the results of short-term genotoxicity assays to tumor incidence, especially when the observed effect is transient, as in the vacuolation of Clara cells, the appearance of ss DNA breaks in mouse liver and lung cells, etc. Dr. Mirer of the UAW [Ex. 126–31] commented,

1. The evidence cited concerns acute effects which appear after a few hours of high level exposure of the animal to methylene chloride vapor, or the glassware (in vitro) mixing of homogenized animal or human tissue with the solvent. In a number of studies the effect in the whole animal is transient.

2. There is no evidence to connect the acute toxic effect, or single strand breaks of DNA after acute exposure, to the chronic effect of lung or liver injury, or cancer. \* \* \*

Dr. Maronpot [Ex. 126–22] was concerned that the vacuolation observed in Clara cells was not reproduced in the NIEHS mechanistic studies. HSIA responded to this concern by remarking that the vacuolation could only be found after single exposures to MC, and that the vacuolation of Clara cells was also associated with increased DNA synthesis in these cells. The fact that this response was only observed after single exposures to MC again raises the issue of the transience of this response and its relevance to MC carcinogenesis.

*Increased cell turnover.*

In these studies on genotoxicity, the authors remarked that increased cell turnover was observed in the lung (transient increase in DNA synthesis after single exposures to MC). Dr. Daniel Byrd [Ex. 126–32] also commented on the DNA synthesis issue. Citing an HSIA study, he contended that there appeared to be a common mechanism of action between the lung and the liver since increased DNA synthesis was observed in both tissues. Dr. Maronpot of the NIEHS [Ex. 126–22] disagreed, stating,

The purported "liver growth" in methylene chloride-exposed mice is actually an increase in liver weight

attributable to accumulation of glycogen within hepatocytes. There is no evidence of replicative DNA synthesis (cell proliferation) in the liver of methylene chloride-treated mice, and, hence, actual increases in the numbers of hepatocytes did not occur. \* \* \* It is noteworthy that recovery to normal liver weight occurs within two weeks after cessation of exposure to methylene chloride.

OSHA agrees with Dr. Maronpot that no data in the rulemaking record show increases in liver cell proliferation as the result of MC exposure, although increased DNA synthesis was actively searched for in the NIEHS mechanistic and other studies. The increased DNA synthesis observed in mouse Clara cells is a transient phenomenon that has not been clearly linked to carcinogenesis in the mouse. In any event, cell proliferation is not necessarily related in any way to carcinogenesis and is often uncorrelated with the doses used in bioassays and the tumor rates themselves. Many substances that cause prolonged cell proliferation do not cause tumor formation and vice versa [Ex. 126–22], and many experts believe that transient increases in cell proliferation, such as seen with MC, cannot account for the carcinogenic effect. Further discussion of cell turnover as a mechanism of carcinogenicity is discussed below under "Non-genotoxic mechanisms."

*Clara cell as the mouse lung tumor cell of origin.*

Another issue raised by commenters concerned the cell of origin of the mouse lung tumors. The mouse lung has a higher proportion of Clara cells than the human lung. The investigators hypothesized that if the Clara cell were the mouse lung tumor cell of origin, the risk estimated from the mouse lung tumor data may overstate human risk because humans have fewer Clara cells, and therefore fewer potential target cells.

Green et al. have focused much of their research efforts into determining the mechanism of action of MC in mouse lung and liver. In lung tissue, as described above, they concentrated on experiments addressing the hypothesis that the mouse Clara cell is the cell of origin of the mouse lung tumors observed in the NTP bioassay. Dr. Daniel Byrd [Ex. 126–32] indicated that he believed that the data presented supported this conclusion. He stated, "Mouse lung tumors most likely arise from damaged Clara cells, although a few pathologists continue to speculate that mouse lung tumors arise from other

lung cells, such as Type II pneumocytes."

In contrast, Dr. Maronpot of the NIEHS [Ex. 126–22] disagreed with that statement, indicating that "\* \* \* current belief among researchers is that mouse lung tumors arise from Type II pneumocytes rather than Clara cells." Dr. Melnick [Ex. 126–33] suggested that the HSIA data are not consistent with the hypothesis that the Clara cell is the tumor cell of origin. He stated,

DNA damage was detected in lungs of mice exposed to 2000 ppm methylene chloride; however, no significant increase in DNA single strand breaks was observed in Clara cells isolated from mice exposed to 4000 ppm methylene chloride. This observation does not support the conclusion that Clara cells were the cells of origin of methylene chloride-induced mouse lung tumors.

In their paper, Graves et al. [Ex. 120] explain their results as follows,

Attempts to measure DNA damage in Clara cells isolated from mice which had been exposed to MC in vivo were unsuccessful. \* \* \* [I]t is possible that cells extensively damaged by MC do not survive the isolation procedure. The observation that the in vivo vacuolation of Clara cells observed after MC treatment is not seen in vitro when the cells are isolated from the damaged lungs supports this proposal.

This means that the authors could induce ss breaks in the DNA of Clara cells *in vitro*, but in mice exposed to MC *in vivo*, it is not clear that the DNA ss breaks observed in lung tissue were concentrated in the Clara cells. In fact, the authors state,

Since Clara cells represent only 5% of the total lung cell population, the DNA ss breaks observed in vivo may not exclusively result from damage to this cell population.

OSHA believes that these issues raise serious doubts as to whether current evidence supports the determination that the Clara cell is the cell of origin of the mouse lung tumors. Although the absence of increased ss breaks is not necessarily an indication of lack of genotoxicity, the presence of ss breaks in lung tissue (and apparently not concentrated in Clara cells) reveals an inconsistency in HSIA's argument: either the ss breaks are irrelevant or Clara cells are not the cells of origin, or both. Further discussion of the issues surrounding identification of the Clara cell as cell of origin for mouse lung tumors is contained below under "Non-genotoxic mechanisms of carcinogenesis."

*Ability of MC reactive metabolites to cross membranes.*

Although no data were presented by the HSIA to address this issue directly, several of the HSIA papers and the accompanying letters postulate that the



reactive metabolites of the GST pathway are too short-lived to cross membranes. This argument is used in combination with the claim of high concentrations of the mRNA for the GST T1-1\* in the nuclei of mouse cells (but not those of rats and humans) to support the contention that humans are not at risk of developing cancer after exposure to MC. The reasoning is as follows: (1) Mice are the only species to have high concentrations of GST T1-1\* in the nucleus of lung and liver cells. (2) The reactive metabolites of the GST pathway are too short-lived to cross the nuclear membrane. (3) In order to produce a carcinogenic effect, reactive metabolites must be produced inside the nucleus in proximity to the DNA. (4) Because the mouse has high concentrations of these enzymes in the nucleus (and rats and humans do not), the mouse is uniquely susceptible to lung and liver cancer after exposure to MC. (5) Therefore, there is no risk of humans developing cancer after exposure to MC.

Some commenters [Exs. 126-12, 126-30, 126-33] maintained that HSIA's submitted studies do not support this argument. As discussed subsequently, the probe used in these experiments measured GST T1-1\* mRNA, not the isozyme itself. There is not necessarily a correlation between the intracellular concentration of mRNA and the concentration of enzyme at a specific locus. In addition, one would expect there to be higher mRNA outside the nucleus (since that is where the enzyme is transcribed from the mRNA), even if the enzyme were subsequently concentrated inside the nucleus. Additionally, as discussed previously, some of the evidence presented by HSIA suggests that the metabolites can be generated outside the cell (not simply outside the nuclear membrane) and interact with the DNA. Specifically, Dr. Dale Hattis [Ex. 126-12] has remarked that,

\* \* \* as long as these reaction and detoxification processes are not infinitely fast (and in principle they cannot be infinitely fast), a finite fraction of the activated metabolite molecules must reach the DNA and react. Even though this chain of events is required by our basic understanding of the relevant kinetic processes, in this case we also have direct empirical evidence that active metabolites need not be generated in a cell's nucleus in order to reach DNA and do damage. The DNA sequence mutations of Graves and Green [Ex. 122] and Graves et al. [Ex. 123], and the DNA single strand breaks reported by Graves et al. [Ex. 120] for CHO cells were all produced by exposing mammalian cells to a tissue culture medium that had been supplemented with mouse metabolizing enzymes and methylene chloride. The active metabolites in those

cases were necessarily generated *from outside of the cells*, not just in the cytoplasm of the cells that manifested the DNA damage. Therefore, the claim that the active glutathione transferase metabolite(s) must be generated in the nucleus and would be ineffective if generated in the cytoplasm is flatly contradicted by HSIA's own evidence.

HSIA [Ex. 126-29] strongly disagreed that their results should be interpreted in this way and countered as follows:

The investigators had to use a suspension assay to maximize the concentration ratio of methylene chloride to cells to about  $10^{14}$ , and to optimize the GST activity from mouse liver preparation. Only under these extreme nonphysiological conditions with a transformed cell line could any increase in mutation frequency be observed. There is absolutely no justification for assuming similar conditions in humans, where GST activity is absent or at very low levels in the cytoplasm and absent in the nucleus.

OSHA disagrees with HSIA, however, and finds Dr. Hattis' and the other commenters' reasoning more sound. The results of these experiments indicate that the metabolites of MC are stable enough to cross the cellular and the nuclear membrane to interact with DNA. The Agency recognizes that these are not physiological conditions, but the conditions of the experiment do support the common-sense assumption that enzymatic metabolism takes place in the cytoplasm of mouse cells and show that some fraction of the GST metabolite(s) is stable enough to cross membranes in the cell. Thus, the Agency believes that the observed tumorigenesis in the mouse is not the exclusive result of nuclear MC metabolism.

#### *Other issues pertaining to genotoxicity.*

The remaining comments on these studies focused on more general issues such as the genotoxicity of MC and other factors related to the GST metabolic pathway and MC-induced carcinogenesis. Dr. Melnick [Ex. 126-33] remarked:

Some fundamental questions related to this mechanism and its uniqueness to mouse liver and mouse lung carcinogenesis are also not addressed by the present research. For example, why do tumors not develop in other organs in mice that also have high levels of GST theta (e.g., kidney)?

OSHA believes this is an important question that reduces the strength of HSIA's contention that the mouse responds in a unique way to MC. The investigators have attempted to explain differences in potency of MC with respect to liver and lung carcinogenesis by invoking differences in DNA repair rates and GST metabolism within the nuclei of critical cells. However, there are other tissues which, based on the

HSIA hypothesis, ought to be prime candidates for carcinogenesis. The kidney, besides having high levels of GST theta, also has a slower rate of DNA repair than the liver. It would appear to be a logical site of carcinogenesis if HSIA's hypothesis is correct. OSHA believes that the lack of tumor response in this organ (and perhaps other logical sites) indicates that the hypothesis proposed by HSIA fails to account for all relevant observations.

(2) *Non-genotoxic mechanisms of carcinogenesis.* Non-genotoxic mechanisms of action have also been hypothesized for MC. Increased cell turnover, due to cell death caused by MC toxicity, could theoretically increase the available number of sites for mutation and subsequent tumor formation. However, there is only limited evidence of increased cell turnover after MC exposure. Casanova and Heck [Ex. 21-16] observed increased DNA synthesis in lung tissue of mice exposed to MC. Green et al. [Ex. 105] observed Clara cell vacuolation, and both studies measured increased DNA synthesis on the first day of exposure to MC, but not on subsequent days of exposure. Clara cells may be targets of MC-induced toxicity because they contain higher levels of MC-metabolizing enzymes and are therefore more likely to generate toxic MC metabolites (for example, carbon monoxide is known to poison MFO enzymes). Green et al. suggested that the Clara cell was the cell of origin of the lung tumors observed in the NTP mouse study, because of the metabolic properties of these cells and the increased cell turnover observed within a day of MC exposure (in addition to the DNA damage described above under the section entitled, "Genotoxic mechanisms of carcinogenesis").

Green et al. further suggested that if the cell of origin of the mouse lung tumors was the Clara cell, humans would be at substantially less risk of lung cancer, because humans have proportionally fewer Clara cells than mice do. However, OSHA believes that there is no clear evidence confirming that Clara cells were the cell of origin of the mouse lung tumors (see discussion above). Other cell types in the lung, such as the Type II lung cell, also have relatively high metabolic activity and could be the site of origin of lung tumors. These cells have not been studied separately. Further studies are needed to clarify the role of the Clara cell and other lung cell types and cells in other tissues in MC carcinogenesis.

(i) *Increased cell division.* In 1994, Foster et al. [Ex. 119] investigated increased cell division as the



mechanism of action of MC in mouse lung cells. Specifically, they examined the mechanism of MC action on the transient vacuolation of bronchiolar cells observed following single exposures to MC. In mice exposed to 2000 and 4000 ppm MC, they observed increased numbers of vacuolated cells in the bronchiolar epithelium. Pretreatment of mice with a cytochrome P450 inhibitor decreased the number of vacuolated cells, while pretreatment with a glutathione depletor did not. In a replication of the observation made by Green et al. and described above, the authors found increased cell division (measured as incorporation of [3H]-thymidine) in Clara cells isolated from mice exposed to 4000 ppm MC. They concluded:

We believe that these results strongly support the supposition that the vacuolation of the Clara cells is due to a toxic metabolite produced by the CYP [cytochrome P-450] pathway of metabolism. Furthermore the most likely candidate for inducing the change is thought to be formyl chloride.

OSHA agrees that these observations indicate that increased cell turnover occurs in Clara cells of mice. This may possibly be a partial explanation of the mechanism, but only a partial one. In cases where cytotoxicity has been considered to be an explanation for risk occurring only at "high" doses, this argument is confined to chemicals believed to act non-genotoxically. MC is likely to be a genotoxic carcinogen, so even if cell proliferation is a factor, the genotoxic mechanism would be the primary mechanism of concern. Genotoxic carcinogens are not generally believed to have a threshold and the dose-response function is believed to be approximately linear at low doses. In addition, the study focused on one type of cell, which may not be the cell of origin for lung tumors. Carcinogenicity in humans (as well as in mice and rats) seems to originate from various cell types in various tissues.

(3) *Metabolism of MC.* As described above, the mechanism of carcinogenesis for MC is not known. Numerous studies over many years have explored numerous possible mechanisms and have provided substantial information regarding the metabolism and the probable metabolite responsible for the carcinogenic effect. As discussed in the Health Effects section, MC is metabolized by two pathways: the mixed function oxidase pathway (MFO) and the glutathione S-transferase (GST) pathway. Both pathways produce reactive intermediates which potentially could contribute to a genotoxic mechanism of carcinogenicity. During

development of the PBPK model for MC, Reitz et al. found that tumor incidence correlated with the estimated amount of GST metabolite, as well as with the amount of parent compound administered, but not with the amount of MFO metabolite [Ex. 7-225]. The parent MC is not likely to act as a genotoxic carcinogen because it is a fairly non-reactive compound. In addition, MC blood levels in mice were lower than in rats, so if MC was the carcinogenic moiety, one would expect the risk of cancer in rats to be higher than mice, whereas the opposite was observed. Consideration of these factors has led many investigators to conclude that the GST pathway is responsible for carcinogenesis and that it is likely to produce a genotoxic carcinogenic moiety. OSHA has reviewed the data available on mechanism of action and has concluded that the most plausible assumption is that the GST pathway is responsible for the carcinogenic action of MC and that this should be taken into account in the quantitative risk assessment. This represents a case-specific departure from the default assumption that the administered dose of the parent compound is the relevant metric for exposure.

(i) *Specific GST isozyme(s) responsible for MC metabolism to the carcinogenic metabolite.* Recent work sponsored by the HSIA was directed at further characterization of the metabolism of MC by the GST pathway [Exs. 121, 124, 124A]. Specifically, the HSIA work on MC metabolism has focused on the isolation and description of isozymes in the GST theta class of enzymes, which HSIA believes are responsible for the metabolism of MC to the carcinogenic metabolite in mice. Mainwaring et al. have shown that the GST isomer with the greatest specific activity for MC is a member of the theta class of GST. [Ex. 121] In rats, three members of the theta class have been identified, GST 5-5, GST 12-12 and GST 13-13. In humans, two theta class enzymes have been identified, GST T1-1 and GST T2-2 and in mice, two theta enzymes have been described, GST T1-1\* and GST T2-2\* (also known as GST MT-1 and GST MT-2). According to Mainwaring et al. [Ex. 121], rat GST 5-5 and mouse GST T1-1\* have similar specific activity toward MC and sequencing studies have shown " \* \* that rat 5-5, mouse T1-1\* and human T1-1 are orthologous proteins as are rat 12-12 and mouse T2-2\* and human T2-2" [Ex. 124A].

The hypothesis under investigation in this work was that the enzyme similar to rat GST 5-5 (mouse T1-1\* and human T1-1) was the critical enzyme

responsible for metabolism of MC to the carcinogenic metabolite, and that differences in the interspecies intra- and inter-cellular distributions of this isozyme and differences in genotoxicity would be important for characterizing the risk of carcinogenesis after exposure to MC.

In order to examine the distribution of the GST isozymes of interest, the investigators used DNA oligonucleotide anti-sense probes complementary to three regions of the protein nucleotide sequences of rat GST 5-5, mouse GST T1-1\* and human GST T1-1 to localize specific mRNA sequences in mouse, rat and human liver and lung tissue. They also used an antibody raised against rat GST 12-12 to localize the protein itself [Exs. 124, 124A]. In the full paper describing these experiments [Ex. 124A], Mainwaring characterized the results of this study, as follows:

The mouse enzymes [T1-1\* and T2-2\*] were present in significantly higher concentrations in both liver and lung than the equivalent enzymes in rat and human tissues. In mouse liver, both enzymes were localized in limiting plate hepatocytes surrounding the central vein, in bile duct epithelial cells and in the nuclei of hepatocytes. In rat liver the distribution of GST 12-12 was comparable to that seen for T2-2\* in the mouse. GST 5-5 was not localized in limiting plate hepatocytes or in nuclei of rat liver. The levels of human transferase T1-1 in the liver were very low, with an even distribution throughout the lobule. The GST 12-12 antibody did reveal high concentrations of this enzyme in human bile ducts. The relative amounts of the theta enzymes in the lungs of the three species followed the pattern seen in the liver, with very high concentrations in Clara cells and ciliated cells of the mouse lung and much lower levels in the Clara cells only of rat lung. Low levels of human transferase T1-1 were detected in Clara cells and ciliated cells found at the alveolar/bronchiolar junction of one human lung sample. The enzyme was entirely absent from the large bronchioles.

Mainwaring et al. concluded that:

This study has demonstrated a highly specific distribution of the theta class GSTs 5-5 and 12-12 in liver and lung tissue from mice, rats and humans. \* \* \* it was apparent from these studies that both the distribution and concentration of these enzymes differed markedly between the three species. Whilst neither mRNA levels nor protein concentrations necessarily correspond to active enzyme, the distribution shown by the mRNA for GST 12-12 was quantitatively reflected by the antibody to the protein of this enzyme, suggesting that these techniques do, in this case, reflect the distribution of active enzyme. Although an antibody to GST 5-5 is not available, it is reasonable to assume that mRNA levels for this enzyme are similarly representative of the distribution of active enzyme.

An understanding of the cellular and sub-cellular distribution of GST 5-5 has provided

an explanation for the species specificity of the mouse lung and liver carcinogen methylene chloride, and has provided reassurance that humans are not at risk from exposure to this chemical.

(ii) *Issues raised pertaining to metabolic studies.* Many commenters commended the HSIA for providing new information on the mechanism of action of MC and for confirming previous quantitative studies of the interspecies differences in MC metabolism. However, commenters also raised several specific issues regarding the conduct and interpretation of these experiments.

*Correlation of mRNA concentrations with enzyme concentrations.*

Mainwaring et al. [Ex. 124A] correlated the inter- and intra-cellular distribution of the mRNA for GST 12-12 in the rat with the distribution of the antibody for GST 12-12. They stated that it is reasonable to assume that since the protein and mRNA for the 12-12 isomer have similar distributions, the protein for the 5-5 isomer would distribute in the same manner as the mRNA for the 5-5 isomer. In support of their assumption, they noted that there is 80% homology between the 5-5 and 12-12 isomer. Some commenters believed that this was not a reasonable assumption and that there was no reason to believe that the distribution of the GST 5-5 isomer protein would correlate with the distribution of the GST 5-5 mRNA simply because there seemed to be a correlation in the 12-12 isomer protein and mRNA distributions [Exs. 126-7, 126-16]. OSHA concurs with these commenters, and until there is actual measurement of the GST 5-5 protein, OSHA does not believe that the question of the actual distribution of GST 5-5 isozyme will have been settled.

More importantly, several commenters stressed that it was mRNA that was actually observed in these studies, and mRNA levels do not necessarily correspond to either protein levels or protein activity within a cell [Exs. 126-7, 126-16, 126-28, 126-30, 126-32]. Although Mainwaring et al. acknowledge this fact [Ex. 124A], the conclusions reached by the authors still suggest that measurement of mRNA is equivalent to measurement of enzyme activity. Referring to the conclusions drawn by Mainwaring et al., Dr. Lorenz Rhomberg [Ex. 126-16] commented:

This interpretation of mRNA distribution is profoundly in error and contradicts some of the most well established and fundamental principles of molecular biology.\* \* \* Finding mRNA in the nucleus is unsurprising and uninformative about the eventual location of the protein products. Detecting mRNA only reveals that the cell may be presumed to be manufacturing the corresponding protein.

Dr. Rhomberg was also concerned that the concentration of GST T1-1\* in the nucleus of mice may be an artifact of the experimental conditions, resulting, perhaps, from a burst of mRNA synthesis. The concern that the apparent nuclear concentration of GST may be an artifact was echoed by Dr. Douglas A. Bell of the National Institute for Environmental Health Sciences [Ex. 126-26]. He stated:

Why the [intracellular] distribution should be different among species is unclear and unusual. Differences in processing of the nuclear RNA transcript from full length pre-mRNA may be the underlying cause of this phenomenon (or perhaps there is a transcribed pseudogene that is complicating the process).

Because of the specific cellular mechanisms that would be required to concentrate a protein in the nucleus, Dr.

Rhomberg [Ex. 126-16] indicated that translocation of the GST 5-5 protein to the nucleus only in mice seemed unlikely. He stated:

It seems implausible \* \* \* that for a series of orthologous proteins, such localization would be found in a particular species and not in other species.

OSHA agrees with the comments made by Dr. Rhomberg and Dr. Bell on this issue, and concludes that the concentration of mRNA at a particular cellular site does not necessarily correlate with concentration of the enzyme itself. OSHA believes that caution should be used when interpreting the results of these experiments.

*Attribution of GST metabolizing activity to a single GST isozyme.*

Concern was also raised about the validity of attributing all of the glutathione S-transferase metabolism of MC to one isomer of the theta class [Exs. 126-7, 126-12]. In particular, Dr. Dale Hattis noted that there was less enzyme activity eluting coincident with the peak identified as the 5-5 form than that eluting at pH 8, which was not believed to correspond to the 5-5 form. Dr. Ronald Brown described results from a paper by Blocki (1994) [Ex. 127-22] which showed that "expression of the [5-5] isozyme contributes 50% of the total GST activity toward this substrate." This leaves the question open as to whether isozymes which may have lower specific activity for MC but which may be expressed in much greater abundance (particularly  $\mu$  4-4), could contribute as much as the remaining 50% of the total GST metabolism (see Table VI-1, reproduced below from Dr. Brown's comment [Ex. 126-7], original source Blocki et al. (1994) [Ex. 127-22]).

TABLE VI-1.—RELATIVE CONTRIBUTION OF DIFFERENT RAT LIVER GLUTATHIONE S-TRANSFERASES IN DICHLOROMETHANE METABOLISM TO FORMALDEHYDE

|  |             |     |     | Glutathione S-transferases |                  |                  |
|--|-------------|-----|-----|----------------------------|------------------|------------------|
|  |             |     |     | $\alpha$ Class             | $\mu$ Class      | $\theta$ Class   |
| Comparative parameter (units) .....                | 1-1+1-2+2-2 | 3-3 | 3-4 | 4-4                        | <sup>b</sup> 5-5 | <sup>b</sup> 13k |
| Specific activity (nmol/min/mg of protein) .....   | <0.1        | 7   | 11  | 23                         | 11,000           | 9                |
| % Cytosolic protein (% of total in liver) .....    | 6.4         | 0.7 | 0.3 | 0.6                        | 0.002            | 0.005            |
| Total activity (nmol/min/g of liver protein) ..... | <10         | 49  | 33  | 138                        | 22               | 0.45             |
| % Total activity <sup>c</sup> .....                | <1.5        | 11  | 7   | 32                         | 50               | 0.1              |

<sup>a</sup> Data from Meyers et al., 1991.

<sup>b</sup> Data for 13,000 molecular weight glutathione transferase from Blocki et al., 1992.

<sup>c</sup> Assuming Vmax conditions for each.

In addition, Mainwaring et al. [Ex. 124A] noted that the "substrate specificity of GST 12-12 is currently poorly characterized," although the purified enzyme has no activity toward

MC. As described above, these enzymes appear to be very labile upon purification. Therefore, it is unclear how much the 12-12 isomer itself may contribute to MC metabolism. As Dr.

Kenneth T. Bogen stated, " \* \* \* while the substrate specificity of GST 12-12 may currently be poorly characterized, current data do not appear to rule out GST 12-12 specificity toward MC."

*Limited human samples and human polymorphism in the GST theta genes.*

Several commenters expressed concern for the limited number of human samples (one pooled lung sample and less than 40 human liver samples have been assayed) and the potential effect of a known human polymorphism for the glutathione S-transferase theta class genes on risk estimations [Exs. 126-7, 126-16, 126-26, 126-35]. Specifically, commenters raised concerns that there may be a large subpopulation of GST conjugators who may be at increased risk from MC exposure that has not been adequately characterized in the limited number of human samples (especially lung samples) that have been tested. HSIA objected to these comments, stating,

The human tissue data base for the metabolism of methylene chloride by the GST pathway is one of the largest, if not the largest, available for this type of risk assessment. To discount it based on arguments concerning hypothetical polymorphisms, as these commenters urge OSHA to do, would be contrary to the message consistently put forward by the National Academy of Sciences and regulatory authorities for the past decade. \* \* \*

In fact, the National Academy of Sciences report cited by HSIA, "Science and Judgement in Risk Assessment" does encourage agencies to make use of biologically-based models, but cautions that using them without adequately considering human variability would be a step backwards:

EPA has not sufficiently accounted for interindividual variability in biologic characteristics when it has used various physiologic or biologically based risk-assessment models. The validity of many of these models and assumptions depends crucially on the accuracy and precision of the human biological characteristics that drive them. In a wide variety of cases, interindividual variation can swamp the simple measurement uncertainty or the uncertainty in modeling that is inherent in deriving estimates for the "average" person. The Academy goes on to recommend specifically that making "reasonable inferences" about interindividual variation is *required*, rather than assuming that no such variation exists:

Even when the alternative to the default model hinges on a qualitative, rather than a quantitative, distinction, such as the possible irrelevance to humans of the alpha-2u-globulin mechanism involved in the initiation of some male rat kidney tumors, the new model must be checked against the possibility that some humans are qualitatively different from the norm. Any alternative assumption might be flawed, if it turns out to be biologically inappropriate for some fraction of the human population.

When EPA proposes to adopt an alternative risk-assessment assumption \* \* \* it should

consider human interindividual variability in estimating the model parameters or verifying the assumption of "irrelevance." If the data are not available that would enable EPA to take account of human variability, EPA should be free to make any reasonable inferences about its extent and impact (rather than having to collect or await such data), but should encourage interested parties to collect and provide the necessary data.

OSHA believes HSIA has misinterpreted the NAS recommendations, and further disagrees with HSIA that the polymorphism is "hypothetical." Investigators have demonstrated this polymorphism in human GST and have shown how the polymorphism varies across races [Exs. 127-7, 127-9, 127-17, 127-21, 127-23, 127-24, 127-25]. OSHA agrees with the commenters that a human polymorphism in the GST theta genes may increase concern for individuals that may be at higher risk from exposure to MC due to their genetic make-up. The Agency has considered sensitive subpopulations in the development of health standards, including this rulemaking. For example, the subpopulation of workers with silent or symptomatic heart disease was considered in assessing the cardiac risks of MC (due to its metabolism to carbon monoxide). The variation in enzyme activity raises additional uncertainty in the use of human data to support the hypothesis that mice are uniquely sensitive to MC carcinogenicity. However, for purposes of quantitative analysis, the Agency has not attempted to systematically adjust the risk estimates based on a "high GST metabolizing" individual because the frequency and impact of such polymorphisms have not been clearly worked out.

*Target site of MC carcinogenesis in mice versus humans.*

Drs. Brown and Melnick [Exs. 126-7, 126-33] also raised the possibility that the target site for MC carcinogenesis may be different in humans than in mice or rats. Specifically, research on the occurrence of theta isomers of GST in human blood was described. The characterization of GST metabolism in human erythrocytes [Exs. 127-11, 127-12] suggests the possibility of the bone marrow as a potential target of MC carcinogenesis and also the potential for metabolism in the blood and translocation of the metabolites to a variety of potential targets. The HSIA discounted human blood metabolism of MC, stating,

The 'very high capacity to conjugate methylene chloride' mentioned by Brown is in fact very low, approximately 40-fold lower

than the highest activity detected in human liver.

OSHA believes that although the specific activity in the blood may be lower than the human liver activity, the total activity of the GST enzymes in blood and marrow may be significant when one also considers the volume of these compartments. OSHA also notes that interspecies tumor site concordance is not necessarily expected, and it is prudent to consider any human tissues which have the potential to metabolize MC to the putative carcinogen.

*Concentration of protein complementary to rat GST 12-12 in human bile ducts.*

Dr. Bogen [Ex. 126-15] commented specifically on the human liver protein complementary to the antibody to rat GST 12-12 protein. In particular he was concerned that high concentrations of this enzyme were reported in bile ducts of the human liver. He noted,

With regard to potential human carcinogenicity of MC relative to its known carcinogenic potential in mice, it seems to me that these particular data ought not to reduce regulatory concern, but rather ought to increase regulatory concern, in view of the fact that bile duct epithelium cells are the most likely stem cells for hepatocytes. \* \* \* Thus hepatocellular bile-duct epithelial cells are likely to play an important role in liver carcinogenesis in both mice and humans.

OSHA agrees with Dr. Bogen's concerns and also notes that in the cohort study of textile workers conducted by Hoescht-Celanese [Ex. 7-260], an excess of biliary cancers was observed in those workers exposed to the highest concentrations of MC and those with the longest latency period between exposure and disease. If the HSIA theory is correct (i.e., a single isozyme is the culprit), then finding high levels of this isozyme in human bile duct is strong evidence implicating MC in human carcinogenesis.

*Interpretation of data as qualitative versus quantitative differences.*

Perhaps most importantly for the purposes of MC risk assessment, several commenters remarked that OSHA should use caution when interpreting the data from the HSIA submissions, because any interspecies differences are rightly considered first as quantitative rather than qualitative ones. In part, the commenters cautioned that one should pay special attention to the threshold of detection in all assays. As Dr. Andrew Salmon stated,

Green and co-workers have consistently confused their inability to measure a result or parameter value due to its magnitude or frequency of occurrence being below their threshold for practical detection, with a true

zero value for the parameter or zero risk of an occurrence [Ex. 126–36].

OSHA agrees that caution should be used when attempting to characterize a difference between species as an absolute qualitative difference. A much higher burden of proof is required to support a claim of zero risk than of diminished risk. (This higher burden is due to the need to consider assay sensitivity and other factors; the fact that the consequences of incorrectly concluding that humans are at zero risk are particularly dire only adds to the already high threshold of scientific evidence needed to successfully make such a claim). In the case of MC, humans clearly have the ability to metabolize MC via the GST pathway [Exs. 21–53, 127–16]. Even if the enzyme concentration of GST T1–1\* itself actually occurs only in the nuclei of mouse lung or liver (as opposed to the concentration of mRNA, which may or may not be localized differently within mouse cells), it is still unclear what impact (if any) this fact would have on the characterization of human cancer risks for MC. OSHA believes that the statement that there are absolute species differences in the activity and intracellular distribution of GST 5–5 is highly speculative and is not supported by the data presented to date, because the data presented refers to the distribution of mRNA for GST 5–5, not the enzyme concentrations or activity levels of the enzyme; there is no quantification of the intracellular levels of the mRNA or enzyme levels, only photographic representations; and there is no evidence that any potential difference in enzyme activity (when those experiments are completed) would be greater than the difference already predicted from allometric scaling considerations.

#### *Conclusions reached by the HSIA.*

HSIA concluded from these studies that because of a qualitative inter-species difference in the distribution of the GST theta enzyme responsible for MC carcinogenesis, humans would not be at risk of developing cancer under “foreseeable conditions of exposure.” Although some commenters agreed with the conclusions reached by the HSIA [e.g., Exs. 126–10, 126–13, 126–20], many commenters strongly disagreed with this interpretation of these data pertaining to the risk assessment for MC. These commenters [e.g., Exs. 126–7, 126–11, 126–12, 126–15, 126–16, 126–22, 126–26, 126–30, 126–36] were concerned that the question was in reality an issue of quantitation of enzyme, not a qualitative difference in

metabolism. Dr. Lorenz Rhomberg commented:

The question is, is there any basis for believing that the species difference in activity suggested by the mRNA data is greater than has been previously supposed?

It should be emphasized that some degree of species difference in metabolic activity is expected even under the default cross-species extrapolation methods. That is, in keeping with the general pattern of scaling of physiological processes across species, general metabolic rates are presumed to be lower on a per unit of tissue basis in larger animals. As a default, this pattern can be presumed to apply to individual metabolic pathways as well, although data on species-species activities can be used in place of such defaults if available.

If species-species activities are discovered by experiment to be less in humans than in mice to the degree already anticipated by allometry, then the experiments are simply confirming the default and no change in the human risk estimates is warranted. If humans have a metabolic activity different than the allometric prediction, the incorporation of such estimates into PBPK models can show different human risks from those predicted under the default. The allometric prediction is that, on a per unit of tissue basis, humans should have about 7-fold lower activity than mice and about 4-fold lower activity than rats.

Given the limit of detection of the assay methods, human metabolic activity (or mRNA levels) only a bit less than the allometric expectation of 7-fold less than mice are often difficult to distinguish from zero. That is, claims that humans have no activity (or no mRNA production) in certain tissues must be judged in the light of the fact that only a small change from the already acknowledged allometric difference can often make the human activity undetectable. A 20-fold mouse-human difference, for example, really only represents a 3-fold exaggeration of the 7-fold allometric pattern, yet many assays may fail to reliably characterize a 20-fold difference as a quantitative difference rather than a qualitative difference.

For the above reasons, claims that human metabolic activity in activating methylene chloride are so low as to be essentially qualitatively different than mice should be interpreted with great caution. In fact, existing assays have great difficulty in detecting species differences in metabolic activity great enough to markedly challenge existing risk assessments.

Another commenter discussed the fact that cellular levels of the GST 5–5 isoenzyme would be expected to be distributed unevenly across cells, putting some cells at greater or lesser risk. This would tend to average out over a tissue and would be best described by tissue metabolism data. Other commenters remarked that there was no need to adjust the risk estimates based on these studies because current pharmacokinetic models already account for interspecies differences in metabolism. Although OSHA has

incorporated data from these studies, especially in its “alternative analysis,” OSHA agrees with Dr. Rhomberg and the other commenters who have taken exception to the HSIA conclusions.

The Agency does not accept the HSIA characterization of the results of the summarized studies. OSHA has determined that no evidence has yet been presented that demonstrates that humans are not at risk of developing cancer after exposure to MC. At most, the presented studies suggest a quantitative inter-species difference in MC metabolism, which was established in previous scientific reports and is already accounted for by PBPK modeling. As discussed extensively in this document, OSHA has concluded that HSIA has undervalued certain strong evidence and has overemphasized some more speculative hypotheses. However, as is clear from this discussion OSHA has carefully considered all of the evidence. Substantial evidence in the record clearly supports OSHA’s conclusions. Consequently, OSHA’s approach of relying on the NTP mouse tumor data as the basis of its quantitative risk assessment continues to be the best approach to risk estimation.

*c. Conclusions regarding the carcinogenesis of MC.* The HSIA submitted these documents to OSHA with a request that the Agency consider the mouse tumor data in light of these additional studies and reject use of the mouse tumor response data as the basis of the Agency’s quantitative risk assessment. OSHA believes it has given proper weight to all the evidence, giving greater weight to that which is of the highest scientific quality. However, in light of HSIA’s request, the Agency reopened the rulemaking record and reviewed all the new data. After submitting these documents for review, the HSIA [Ex. 126–29] remarked on comments submitted to the docket by other scientists,

In general, the comments submitted by R. Maronpot, R. Brown, L. Rhomberg, K. Bogen and D. Hattis exhibit a reluctance to use the large body of mechanistic data now available in assessing the potential carcinogenic risk posed by methylene chloride, even though most other commenters agree that the pathway responsible for its observed carcinogenicity in mouse liver and lung, as well as species variations in activity of this critical pathway, have now been identified. Much of the comment addressed here appears to be motivated by a desire to maintain the “status quo” for assessing carcinogenic risk based on default principles that were developed twenty years ago.

The HSIA goes on to say,

Many of the conclusions reached by the commenters \* \* \* are based, often erroneously, on single aspects of one or the other of these publications, rather than on the entire data base, as a "weight of evidence" approach would demand and as is necessary to understand the results.

OSHA finds it difficult to understand why HSIA believes that the scientists they listed are primarily interested in preserving the "status quo." Dr. Maronpot conducted the mechanistic studies on MC at NIEHS, which have generated mechanistic information useful to the risk assessment process. Dr. Rhomberg was instrumental in developing the pharmacokinetic approach used by the Environmental Protection Agency in its risk assessment of MC (an approach never used by the Agency previously). Dr. Hattis, Dr. Bogen and Dr. Brown are all experts in the application of pharmacokinetic modeling to risk assessment and have repeatedly called for incorporating more mechanistic and physiological data into pharmacokinetic models. These highly respected scientists, among others, reviewed the HSIA submissions critically and independently and reached conclusions different from those of the HSIA, conclusions which themselves depart significantly from the "status quo." This does not suggest to OSHA that they are trying to preserve some status quo in risk assessment, and OSHA finds nothing in the comments of these experts to suggest that this is the case.

In order to respond to HSIA's desire to have OSHA further review all of the data, the Agency has reviewed each submitted study carefully and critically on its own merits to determine how each piece of data fits into the overall picture of the mechanism of action for MC. OSHA believes that in this process the critical issues raised by the HSIA have received a full airing and the hazard identification and the risk assessment for MC have been improved because of it. OSHA believes, however, that looking *only* at the new studies submitted by HSIA, and examining them uncritically, would contradict every principle of scientific analysis.

In summary, in order to accept the HSIA's supposition that MC is not carcinogenic in humans, one must believe the following:

1. GST 5-5 is the only isozyme which can metabolize MC to the carcinogenic metabolite.

2. DNA single strand breaks are relevant and a sufficient measure of the tumorigenicity of a compound.

3. The absence of detectable increases in DNA ss breaks in a single experiment

means that there are in fact no additional ss breaks.

4. The limited number of human samples (one sample of pooled lung tissues being the absolute extreme of "limited" data) used to determine metabolic parameters are truly representative of the range of human variability.

5. An apparent correlation in the distribution of the GST 12-12 protein and GST 12-12 mRNA means that the distribution of GST 5-5 protein will correlate similarly with the distribution of GST 5-5 mRNA.

6. Visual interpretation of photomicrographs staining for GST mRNA gives a true and accurate measure of GST activity in the cell.

And one must also ignore the following contradictory observations and conclusions about the mechanism of action (in addition to ignoring the suggestive epidemiologic evidence):

1. Metabolites of GST can cross cell and nuclear membranes and interact with DNA to induce DNA ss breaks and mutations.

2. GST mRNA and protein stain heavily in human bile duct cells (believed to be precursors of hepatocytes).

3. Human lung tissue has been shown to stain for GST mRNA.

4. Only 50% of the GST metabolism of MC can be accounted for by the GST 5-5 isozyme.

5. The metabolic capacity of GST 12-12 for MC has not been characterized.

OSHA concludes that these studies, even putting aside all technical objections to the methodology and interpretation of individual studies, do not change the conclusion that substantial evidence supports the carcinogenicity of MC. The bioassay results in mice are still qualitatively and quantitatively relevant to humans. Once the HSIA studies have been replicated and key components quantified (like the intracellular enzyme activity (instead of mRNA levels) of GST towards MC), the HSIA data may be useful in characterizing quantitative interspecies differences in MC GST metabolism. In particular, it would be useful to determine whether all of the evidence that HSIA submitted is consistent with an allometric difference (a difference expected based on the size of the animal) in sensitivity to MC or with a greater interspecies difference in sensitivity. (The specific activity of GST toward MC in mice is estimated to be about 7-fold that of humans, based on allometric considerations.) OSHA believes that its final risk assessment, which relies on an analysis of all

available PBPK data, addresses both possible interpretations.

## *B. Selection of Database for Quantitative Risk Assessment*

### *1. Animal Bioassays*

The first step in performing a quantitative assessment of carcinogenic risk based on animal data is to choose a data set or sets from which to define the dose-response relationship. In its NPRM, OSHA had chosen the NTP female mouse lung and liver tumors to determine its estimates of risk. OSHA chose these responses because they provided clear dose-response relationships, had low background tumor rates and were more sensitive measures of dose-response than corresponding male mouse tumor sites.

The EPA, the CPSC and the FDA chose to use the combined incidence of adenomas and carcinomas of the lung and liver as the basis for their risk assessments. Specifically, the EPA [Exs. 25-D, 28] placed emphasis on the experimental species and sex group showing the highest risk: the number of female mice showing either adenoma or carcinoma in either lung or liver (or both). The CPSC [Ex. 25-I] pooled benign and malignant tumors of either the mammary gland, lung or liver and averaged male and female estimates to derive an overall risk estimate. The FDA [Ex. 6-1] used benign and malignant responses of female mice. The Crump report [Ex. 12] noted that it may be reasonable to combine lung and liver responses to give an indication of the potency of MC, due to the fact that metabolism of MC occurs by the same pathway in both lung and liver and thus results in the same ultimate metabolites. However, the report added that since both tissues have different background responses, combining responses may tend to complicate the interpretation of risk estimates.

In OSHA's final rule, the NTP study (rat and mouse, inhalation) was chosen for quantitative risk assessment because it provided the best toxicological and statistical information on the carcinogenicity of MC [Exs. 12, 7-127] and because the study was of the highest data quality. In the NTP study, MC induced significant increases both in the incidence and multiplicity of alveolar/bronchiolar and hepatocellular neoplasms in male and female mice. In rats, dose-related, statistically significant increases in mammary tumors were also observed. OSHA chose the female mouse tumor response as the basis of its quantitative risk assessment, because of the high quality of data, the clear dose response of liver and lung

tumors and the low background tumor incidence. Although the female rat mammary tumor response was also dose-related, the data of high quality and amenable to quantitative risk assessment, the mouse data set had a clearer dose-response in both liver and lung tumors than the rat mammary tumor response and the mouse background tumor incidence was lower than in the rat. Therefore the mouse data set was chosen for quantitative analysis.

OSHA included the lung adenomas in the quantitative analysis. The evidence suggests that the presence of benign tumors with the potential to progress to malignancies should be interpreted as representing a potentially carcinogenic response. This belief is supported by the OSTP's views on chemical carcinogenesis (50 FR 10371). OSTP stated that at certain tissue sites, such as the lung, most tumors diagnosed as benign really represent a stage in the progression to malignancy. Additionally, NIOSH, the EPA, the CPSC and the FDA have also included benign responses in their assessments. Therefore, it is appropriate and sometimes necessary to combine certain benign tumors with malignant ones occurring in the same tissue and the same organ site. In particular, OSTP also stated that "the judgement of the pathologist as to whether the lesion is an adenoma or an adenocarcinoma is so subjective that it is essential they be combined for statistical purposes." (50 FR 10371).

OSHA chose female mouse lung tumors as the specific tumor site for its final quantitative risk assessment. There is no *a priori* reason to prefer the mouse lung tumor response over the liver tumor response, because both data sets were of high quality, showed a clear dose-response relationship and had low background tumor incidence. In fact, in the NPRM, the Agency reported estimates of risk generated using both sites. However, to reduce the complexity of the final PBPK analysis, which required highly intensive computations, OSHA chose one site (the female mouse lung tumor response) for its final risk estimates. The risks calculated using the female mouse liver response would likely be slightly lower than those calculated using the lung tumor response. On the other hand, pooling the total number of tumor-bearing animals having either a lung or liver tumor (or both) (which is the procedure EPA advocates [see its 1986 Guidelines for Cancer Risk Assessment]) would have yielded risk estimates higher than OSHA's final values.

The NTP study has been described in the Health Effects section and, above, in the discussion regarding hazard identification.

## 2. Epidemiologic Data

The epidemiology data are not as useful for quantitative risk assessment as the animal data because the animal data provide a clear dose-response, with fairly precise indices of exposure, which cannot be derived from the epidemiology data. All other things being equal, risk assessors would prefer to use epidemiologic data to assess cancer risk in humans over data from animal studies whenever good data on human risk exist. However, the uncertainty inherent in epidemiologic studies must be accounted for; in particular, "positive" studies often have lower confidence limits that do not rule out the no-effect hypothesis, while ostensibly "negative" studies often have UCLs that would support a substantial positive effect. OSHA believes (see discussion below) that the latter circumstance applies to some of the MC studies. Other factors, such as duration and intensity of a chemical exposure (which can rarely be controlled and accurately measured in an epidemiological study), difficulty in accurately defining the exposed population, and other confounding factors diffuse a study's predictive power of true risks.

Frequently, animal studies indicate a positive response to a particular chemical when epidemiologic studies of exposures to the same chemical fail to exhibit a statistically significant increase in risk. When animal studies show a substance to be a carcinogen but epidemiologic studies are non-positive, the minimum risk which could be detected by the human study should be estimated to assess the strength of the epidemiologic study and justify its importance in the risk assessment process. Similarly, the animal-based potency estimate can be used to predict the number of human deaths investigators would likely have seen in an epidemiologic study if the animal-based estimate was correct; if the observed number of human deaths is markedly inconsistent with this predicted number, the relevance of the animal-based estimate might well be called into question. If the human data are equivocal, or the epidemiologic study is not sufficiently sensitive to identify an increased risk predicted by a well-conducted animal bioassay, it is necessary to consider the animal data to protect workers from significant risk. OSHA concludes that the MC epidemiology studies do not have

adequate information upon which to base a quantitative risk assessment. OSHA has, however, used the analyzed epidemiological data to determine whether the results are consistent with those estimated using the rodent models. This is discussed later in the document.

## 3. Conclusions

After reviewing the animal data and the quantifiable epidemiology data, OSHA has determined that the NTP female mouse lung tumor response is the appropriate data set on which to base its quantitative risk assessment, and has determined that the most scientifically-appropriate way to use these data involves constructing a PBPK model to extrapolate from animals to humans. OSHA believes that the non-positive epidemiology data, in particular those from Kodak, are of insufficient power to rule out the risk estimates derived from the animal data.

### C. Choice of Dose-Response Model

Several approaches have been used to estimate cancer risk from exposure to toxic agents. A standard approach uses mathematical models to describe the relationship between dose (airborne concentration or target tissue dose surrogate) and response (cancer). Generally, mathematical functions are fit to the data points observed at different exposure levels and these functions are used to estimate the risk that would occur at exposure levels below those observed. The shapes of these curves vary, ranging from linear extrapolations from the observed points through the origin (zero exposure and zero risk) to curves which may deviate far from linearity at the very highest or lowest doses. The use of a particular model or curve can be justified in part by statistical measures of "goodness-of-fit" to observed data points. That is, there are various statistical tests which measure how closely a predicted dose-response curve fits the observed data.

The most commonly used model for low-dose extrapolation is the multistage model of carcinogenesis. This model, derived from a theory proposed by Armitage and Doll in 1961, is based on the biological assumption that cancer is induced by carcinogens through a series of independent stages. The Agency believes that this model conforms most closely to what we know about the etiology of cancer. There is no evidence that the multistage model is biologically inappropriate, especially for genotoxic carcinogens, which MC most likely is. The most recent data submitted by the HSIA [Exs. 117-124A] clearly add substantial support to the previous body

of evidence indicating that one or more metabolites of MC is a genotoxic carcinogen. The low-dose linearity feature of this model is scientifically required for any exposure that confers additional risk upon a pre-existing background level of risk produced by a similar or equivalent mechanism. Given the underlying connection between DNA mutations and cancer and the obvious background incidence of cancer in the human population, the overwhelming scientific consensus is that genotoxins follow low-dose linear functions.

The multistage model is generally considered to be a conservative model because it is approximately linear at low doses and because it assumes no threshold for carcinogenesis, although there are other plausible models of carcinogenesis which are more conservative at low doses. "No threshold" means that any incremental amount of exposure to a carcinogen is associated with some amount of increased risk. "Approximately linear at low doses" means that one unit of change in dose will result in one unit of change in risk at low doses.

The most common approach for setting the parameters in the multistage model is to assume that the dose-

response curve is described by a polynomial of  $k-1$  degrees, where  $k$  is the number of dose groups tested. The multistage model thus takes the form  $P(\text{Cancer}) = 1 - \exp(-f(\text{dose}))$ , with  $f(\text{dose})$  given by:

$$f(\text{dose}) = a + b_1(\text{dose}) + b_2(\text{dose})^2 + \dots + b_{k-1}(\text{dose})^{k-1}.$$

The number of stages is specified by  $k-1$ , and the parameters  $a$  (the background risk) and  $b_i$  are estimated from the observed data.

Alternatives to the multistage model include the tolerance distribution models such as the probit model, the logit model and the Weibull model. The tolerance distribution models generally predict dose-response relationships which are sigmoid in shape. Thus, these models will approach zero more rapidly than a linear multistage model. This means that at low doses, these models will predict lower risks than will a linear multistage model.

In the MC rulemaking, most of the risk assessments submitted to the Agency used the linearized multistage model to predict risk. The differences in risk estimates were not generally due to the dose-response model used, but to whether the risk assessor used pharmacokinetic modeling to estimate

target tissue doses, and what assumptions were used in the pharmacokinetic modeling.

#### D. Selection of Dose Measure

##### 1. Estimation of Occupational Dose

The purpose of low dose extrapolation is to estimate risk of cancer at a variety of occupational exposures. This requires that the doses be converted into units comparable to those in which the experimental dose is measured.

In its NPRM, OSHA first converted the experimental dose, measured in ppm, to an inhaled dose, measured in mg/kg/day. The female mouse body weight used in these calculations was 0.0308 kg. The breathing rate for mice was 0.05 m<sup>3</sup>/day. The Agency then assumed that equivalent doses in mg/kg/day would lead to equivalent risk. Once the experimental dose (in mice) had been converted to mg/kg/day, it was then converted to ppm using the human breathing rate of 9.6 m<sup>3</sup>/workday and human body weight of 70 kg in order to estimate risks at various potential exposure levels. To determine the dose to humans corresponding to the risk estimated from the mouse data, OSHA used the following equations:

$$\text{Dose}_M(\text{mg/m}^3) = \frac{\text{Dose}_M(\text{ppm})(84.9\text{g/mol})(1000\text{ mg})(1000\text{ L})}{24.45\text{ L/mol}(\text{g})(\text{m}^3)}$$

$$\text{Dose}_M(\text{mg/kg/d}) = \frac{\text{Dose}_M(\text{mg/m}^3)(0.05\text{m}^3/\text{d})(6\text{hr}/24\text{hr})(5\text{d}/7\text{d})}{(0.0308\text{ kg BW})}$$

OSHA assumed that risk estimates derived for mice at a given mg/kg/d would be equivalent to risks

experienced by humans at that mg/kg/d. Doses in mg/kg/d in humans were converted to ppm to determine risk at

various potential workplace exposures using the following equations:

$$\text{Dose}_H(\text{mg/m}^3) = \frac{\text{Dose}_H(\text{mg/kg/d})(70\text{kg})}{(9.6\text{m}^3/\text{workday})(5\text{d}/7\text{d})(45\text{yr}/70\text{yr})}$$

$$\text{Dose}_H(\text{ppm}) = \text{Dose}_H(\text{mg/m}^3)(24.45\text{L/mol})/(84.9\text{ g/mol})$$

This process was used by K.S. Crump et al. in their risk assessment submitted to OSHA [Ex. 12]. Use of mg/kg/d as a measure of dose has been criticized by Mr. Harvey Clewell, representing the U.S. Navy [Ex. 19-59]. He stated,

Strictly speaking, the concept of a mg/kg/day dose applies only to exposures for which the term "administered dose" is well defined, which does not include inhalation exposure to a volatile, lipophilic chemical such as MC....If a non-pharmacokinetic dose surrogate is desired, the choice should be

time-weighted average concentration (ppm) as used by the FDA.

Mr. Clewell preferred use of dose surrogates calculated in the PBPK models to estimate human risk. OSHA has given careful consideration to the issues raised by Mr. Clewell and, in the risk assessment presented here, considered dose surrogates estimated in PBPK models and time-weighted average concentration in addition to the mg/kg/d dose presented in the NPRM.

For all dose measures used to estimate human risk, the assumptions used by OSHA for body weights and exposure times and rates were those described above. In OSHA's final risk assessment, a Bayesian analysis was used and the prior distribution for breathing rate was centered on OSHA's preferred value of 9.6 m<sup>3</sup>/d.



## 2. mg/kg/d Versus Other Measures of Exposure

Quantitative risk assessments based on animal data are conducted under the assumption that animals and humans have equal risks from lifetime exposures to a chemical when exposure is measured in the same unit for both species. Opinions vary, however, on what is the correct measure of exposure. For site-of-contact tumors, a ppm-to-ppm conversion is a generally accepted measure of dose. For systemic tumors, commonly used dose conversions include mg/kg/day (as used by OSHA in its MC NPRM), mg/surface area/day (with surface area approximated by  $BW^{2/3}$ ), mg/ $BW^{3/4}$ /day, and mg/kg/lifetime. When adequate and appropriate pharmacokinetic or metabolic data are available, these data are sometimes used to estimate internal dose. In the case of MC, metabolic data have been gathered and pharmacokinetic models have been used by various investigators to estimate target tissue doses for MC.

Some commenters [Exs. 19–28, 19–57] had expressed concern that OSHA used a surface area correction factor in its risk assessment in the NPRM. In fact, in the NPRM, OSHA extrapolated from mice to humans based on body weight rather than surface area. However, the Agency requested comment on which species conversion factor would be appropriate to use in OSHA's final risk assessment and whether incorporation of pharmacokinetic information should influence the choice of the conversion factor. Two commenters [Exs. 19–83, 23–21] referred to the interagency document on interspecies scaling which ultimately recommends  $BW^{3/4}$  as the appropriate extrapolation factor in the absence of appropriate pharmacokinetic information, although the document also indicates that extrapolation factors based on BW or  $BW^{2/3}$  would also be consistent with the available data (EPA Draft Report: "A cross-species scaling factor for carcinogen risk assessment based on equivalence of mg/kg<sup>3/4</sup>/day." 57 FR 24152, June 5, 1992).

There was also considerable discussion as to whether it was appropriate to apply an extrapolation factor such as  $BW^{3/4}$  or  $BW^{2/3}$  in addition to PBPK modeling of dose, to account for pharmacodynamic differences between species (such as differences in DNA repair rates and other non-metabolic differences in interspecies susceptibility to an agent). The EPA applied the  $BW^{2/3}$  extrapolation factor after incorporation of the PBPK data for MC in their 1987 draft update of the MC risk assessment.

In their previous risk assessment, which did not incorporate PBPK data, EPA also used  $BW^{2/3}$  as the extrapolation factor. Since OSHA has preferred the BW extrapolation in other chemical-specific risk assessments and has used BW as the extrapolation factor in its best estimate of risk in the NPRM for MC, OSHA agrees with Dr. Lorenz Rhomberg's assessment [Ex. 28] that OSHA should continue to use body weight as its extrapolation factor in its final MC risk assessment. Thus, OSHA's risk estimate does not make any allowance for possible pharmacodynamic differences between rodents and humans, or within the diverse human population.

## 3. Pharmacokinetic Modeling of Dose

OSHA discussed issues relating to the use of pharmacokinetic data in its NPRM. These issues were further explored during the hearings and in pre-hearing and post-hearing comments. In response to the ANPR [51 FR 42257], Dow Chemical submitted documentation of a physiologically-based pharmacokinetic model (PBPK) [Exs. 8–14d and 10–6a], developed for MC by Reitz and Anderson, which described the rates of metabolism of the MFO and GST pathways and the levels of MC and its metabolites in various tissues of rats, mice, hamsters and humans. This model was presented as a basis for converting an applied (external) dose of MC to an internal dose of active metabolite in the lung and liver in various species under various MC exposure scenarios. Since publication of the NPRM, several parties have submitted pharmacokinetic models or comments on modeling to the rulemaking record. These are discussed in detail below.

a. *General issues in PBPK modeling.* Physiologically-based pharmacokinetic modeling can be a useful tool for describing the distribution, metabolism and elimination of a compound of interest under conditions of actual exposure and, if data are adequate, can allow extrapolation across dose levels, across routes of exposure and across species. One limitation of using PBPK modeling is a widespread lack of adequate and appropriate physiological and metabolic data to define the model. In particular, difficulties arise in attempting to define a model for which the mechanism of carcinogenesis has not been established, when it is unclear whether there would be tumor site concordance across species, and when the metabolic pathway(s) responsible for carcinogenesis has not been determined.

The concentration of a chemical in air or the total inhaled dose (mg/kg/d) may

not be the most biologically relevant dose to use in comparing toxicity across doses or across species. The dose measure that would be most useful in risk assessment is the dose to the target tissue of the chemical or metabolite that is known to directly cause the toxic effect. Generally, this quantity is unknown in almost every case because the proximate carcinogenic moiety is usually highly reactive, and therefore very difficult to measure in biological systems. Since the proximate toxic agent is unlikely to be a quantity readily measured in the laboratory, it is sometimes desirable to use dose surrogate concentrations, calculated by methods such as PBPK modeling, to obtain a more direct estimate of a dose-response relationship. Examples of dose surrogates that may be relevant to the toxic mechanism of action of a chemical are peak concentrations of a particular metabolite at a target tissue site, area under the concentration-time curve of a metabolite at a target site, and blood concentration of the parent chemical or a relevant metabolite.

If the dose surrogate chosen is directly relevant to the mechanism of action of a chemical, there is greater confidence in the risk estimates generated using the dose surrogate than those generated using total inhaled concentration. If the mechanism of action of a chemical is uncertain, and therefore the relevance of the dose surrogate to carcinogenicity is in question, there is proportionally less confidence in the predicted risks estimated using that dose surrogate. Risk estimates from PBPK modeling can also be limited by the quality and quantity of available metabolic data. Since risk estimates are directly dependent upon the dose or dose surrogate chosen, reliable measures of all relevant physiological parameters and all relevant metabolic pathways in all target tissues from all species under investigation are critical. In addition, measures of the uncertainty and inter-individual variability of these parameters must be generated.

In its NPRM, OSHA solicited information on the appropriateness of physiologically-based pharmacokinetic modeling for the MC risk assessment. Specifically, OSHA asked the following questions:

(a) How can pharmacokinetics be best applied to the risk assessment of MC and what are the current limitations of this approach in the quantitation of health risks? What weight should OSHA give to pharmacokinetic data in its risk assessments and why?

(b) Given that five separate risk assessments have utilized the pharmacokinetic models for MC in five

different ways (resulting in from 0 to 170 fold reduction in the final risk when compared with assessments not utilizing pharmacokinetic data), how can OSHA best utilize the existing pharmacokinetic data and still be certain of protecting worker health?

(c) Which parameters in the pharmacokinetic models are most sensitive to errors in measurement or estimation? Can an increased database reduce the uncertainties in these parameters?

(d) How much confidence can be placed in the human *in vitro* MC metabolism data, especially that for lung tissue? How will human variability in these parameters affect the extrapolation of risk from rodent species?

(e) Are there any studies in progress which attempt to verify the predictive ability of the model *in vivo*, (e.g., by giving doses in a lifetime bioassay which will produce cancer in a species other than the B6C3F1 mouse and the F344 and Sprague-Dawley rats)?

(f) OSHA recognizes the large areas of uncertainty which exist in applied dose risk assessment procedures. If pharmacokinetic modeling reduces these uncertainties, can the reduction in uncertainty be quantified? Are additional uncertainties introduced into the risk assessment process by the use of pharmacokinetic models?

(g) By using the pharmacokinetic models in the risk assessment process, one is making an assumption about the carcinogenic mechanism of action of methylene chloride. Are there any new studies on the carcinogenic mechanism of action of MC which would support or refute this assumption?

(h) If the carcinogenic process is, in fact, not the result of the metabolite(s) from the GST pathway alone, but is due to a combination of metabolites or a combination of the parent compound plus the metabolites, how would the pharmacokinetic model and the subsequent risk assessments be affected? Can these effects be quantified?

(i) One of the assumptions made in the pharmacokinetic model is that the target tissues for MC are liver and lung. Can this model predict cancer incidences at other sites? If not, is there a way to factor in consideration of possible MC-induced human cancers at other sites than liver and lung?

(j) OSHA solicits information supporting or refuting interspecies allometric scaling based on body weight or body surface area.

OSHA reviewed comments and testimony on these issues from an expert witness [Ex. 25-E]; representatives of other U.S. government agencies, including NIOSH

[Exs. 19-46, 41], EPA [Exs. 25-D, 28], CPSC [Ex. 25-I] and U.S. Navy [Exs. 19-59, 96]; the State of California [Ex. 19-17]; the Halogenated Solvents Industry Alliance (HSIA) [Exs. 19-45, 19-83, 105]; and the UAW [Exs. 19-22, 23-13, 61]. Comments and testimony from the expert witness, the other government agencies and the Halogenated Solvents Industry Alliance generally reflected the opinion that the pharmacokinetic information was sufficiently developed in the case of MC to justify its use in estimating human cancer risks. The predominant view among these commenters and hearing participants was that the data collected for MC and the pharmacokinetic model developed by Reitz and Andersen adequately represented the metabolism of MC in mice. Many commenters also believed that it was reasonable to conclude that the lung and liver tumor incidence in the B6C3F1 mice was the result of the GST metabolite. As described in further detail below, OSHA generally agrees that the PBPK approach is reasonable to assess cancer risks of MC. In fact, the Agency has evaluated the submitted PBPK models, determined that there were several deficiencies in each of those models, and improved upon those in its final quantification of risks.

One rulemaking participant was strongly opposed to using pharmacokinetic data in the MC risk assessment. Dr. Franklin Mirer [Ex. 61], representing the UAW, stated:

The pharmacokinetic model advanced for methylene chloride carcinogenesis is incorrect and should not be used for quantitative risk assessment.

Dr. Mirer was particularly concerned that the PBPK model ignored the rat cancer bioassay data and that the model was based on a "mechanistic hypothesis."

Dr. Mirer reiterated his concerns in response to the October 24, 1995 reopening of the rulemaking record [Ex. 126-31], stating,

The simple message is that OSHA should give no additional weight to the pharmacokinetic argument. For OSHA to give the argument any additional weight would mean that OSHA was ignoring a substantial body of evidence regarding carcinogenicity of methylene chloride in additional animal species.

Dr. Mirer continued,

The pharmacokinetic hypothesis is unconvincing even as an explanation of the differences in lung and liver tumors in mice and rats.

OSHA shares Dr. Mirer's concerns that the mechanism of carcinogenicity for MC has not been clearly established and that using pharmacokinetic

modeling may lead to risk estimates which ignore the rat tumor data. The Agency notes that it has used the NTP rat data in its hazard identification for MC. OSHA has also determined, however, that the mouse data represent the strongest data set on which to base a quantitative risk assessment, and notes that risk estimates based on the rat data (without PBPK-based adjustment of dose) are similar to OSHA's final risk estimates using mouse data and a PBPK analysis.

The determination that the mouse data set was the strongest on which to base a quantitative risk assessment was made without regard to the availability of information on pharmacokinetics. Incorporating pharmacokinetic modeling into the risk assessment for MC is a logical extension of OSHA's risk assessment decisionmaking process and reflects the Agency's review of the totality of data on tumor incidence, metabolism and mechanism of action. The extensive data base on MC metabolism and mechanism of action, although by no means complete, was the determining factor in the decision to incorporate pharmacokinetics into its final risk assessment. The Agency is aware of very few chemicals of regulatory interest for which the available data could match this body of information. The specific criteria utilized by the Agency in making this determination are enumerated below.

Comments on the specific issues enumerated above are discussed under the appropriate topics in the sections that follow.

**b. Criteria for using PBPK in quantitative risk assessment.** OSHA evaluated several criteria before deciding to use PBPK analysis in its final quantitative risk assessment for MC. In future rulemakings in which the use of pharmacokinetic information in risk assessment is at issue, it will be necessary to evaluate at least the criteria described below before reaching conclusions, in order to avoid adopting an alternative hypothesis that is less (rather than more) reflective of the true situation than the more generic applied-dose assumption. Further, it may be appropriate to evaluate additional criteria in some cases, depending on the metabolism and mechanism of action of the chemical. The criteria which OSHA considered before incorporation of PBPK in the final risk estimate for MC were:

(1) The predominant and all relevant minor metabolic pathways must be well described in several species, including humans. (Two metabolic pathways are responsible for the metabolism of MC in humans, mice, rats and hamsters).

(2) The metabolism must be adequately modeled (Only two pathways are responsible for the metabolism of MC as compared to several potential routes of metabolism for other compounds, such as benzene and the dioxins. This simplified the resulting PBPK models).

(3) There must be strong empirical support for the putative mechanism of carcinogenesis (e.g., genotoxicity) and the proposed mechanism must be plausible.

(4) The kinetics for the putative carcinogenic metabolic pathway must have been measured in test animals *in vivo* and *in vitro* and in corresponding human tissues (lung and liver) at least *in vitro*, although *in vivo* human data would be the most definitive.

(5) The putative carcinogenic metabolic pathway must contain metabolites which are plausible proximate carcinogens (for example, reactive compounds such as formaldehyde or S-chloromethylglutathione).

(6) The contribution to carcinogenesis via other pathways must be adequately modeled or ruled out as a factor. For example, there must be a reasonable analysis of why reactive metabolites formed in a second pathway would not contribute to carcinogenesis (e.g., formyl chloride produced via the MFO pathway is likely to be too short-lived to be important in MC carcinogenesis).

(7) The dose surrogate in target tissues (lung and liver in the case of MC) used in PBPK modeling must correlate with tumor responses experienced by test animals (mice, rats and hamsters).

(8) All biochemical parameters specific to the compound, such as blood:air partition coefficients, must have been experimentally and reproducibly measured. This must be true especially for those parameters to which the PBPK model is most sensitive.

(9) The model must adequately describe experimentally measured physiological and biochemical phenomena.

(10) The PBPK models must have been validated with data (including human data) which were not used to construct the models.

(11) There must be sufficient data, especially data from a broadly representative sample of humans, to assess uncertainty and variability in the PBPK modeling.

In the case of MC, to a large extent these criteria were met. This made evaluation of existing PBPK models and further development of the modeling strategy a viable option. Therefore, the Agency evaluated existing PBPK models

and then contracted with Drs. Andrew Smith, Frederic Bois, and Dale Hattis to help OSHA improve on the MC PBPK model in the record, which would extend the application of modeling techniques beyond those models which had been submitted to the Agency and incorporate all of the data available and appropriate for quantitative analysis in the record. OSHA's evaluation of existing PBPK models, the development of a modified MC PBPK analysis, and OSHA's final risk assessment are described later in this document.

c. *Choice of GST metabolic pathway as dose surrogate.* The choice of "dose surrogate" for the MC PBPK model is a critical factor in estimating PBPK-based risks. The dose or "dose surrogate" used in a risk assessment should be a biologically-important quantity, should have a plausible mechanism of action at the target tissue and should correlate with the response of interest. The simplest choice of dose is the applied dose or ambient concentration of the contaminant measured as ppm or as the inhaled quantity in mg/kg/day (as used in the Preliminary Quantitative Risk Assessment in the NPRM). Such quantities have the advantage of being easily and directly measurable during the bioassay. Other meaningful dose surrogates could include the concentration of parent compound in the target organ, the concentration of specific metabolites in the target organ, the area under the time-concentration curve (integrated dose) of each metabolite and the parent compound, or peak blood or target organ levels of each metabolite and parent compound. These quantities are not as easily measured. Often only indirect measurements or computer modeling of these dose surrogates are available.

In the PBPK model developed by Reitz et al. [Ex. 7-225], the dose surrogates that correlated with the tumor response were the parent compound (MC) concentration and the amount of GST metabolites formed in the lung and liver. Reitz et al. discounted the parent compound as the dose surrogate because MC is not a chemically reactive compound and direct-acting carcinogens (and metabolites of carcinogenic compounds) are generally hypothesized to be reactive (usually, electrophilic). They also discounted the parent compound as a relevant dose surrogate because parent MC concentration was higher in the rat blood than in the mouse for any dose of MC, while the cancer response of the mouse was greater than the rat. If parent MC were the critical compound for MC carcinogenesis, one would expect the cancer response across species to

correlate with blood levels of the compound.

(1) *Metabolism via GST versus MFO pathway.* Human metabolism of MC has been well studied. One clear finding from the human metabolic studies is that humans metabolize MC by both the MFO and GST pathways, as do mice, rats, and hamsters. Although human metabolism via the MFO pathway has been measured *in vivo* as well as *in vitro*, human MC metabolism via the GST pathway has been measured only *in vitro*. Metabolic data on the human GST pathway have been collected from several liver samples and one pooled lung sample (combined samples from four human subjects). However, it has not been possible to measure human GST metabolism of MC *in vivo*.

Reitz et al. measured the metabolic constants ( $K_m$  and  $V_{max}$ ) *in vitro* for the GST and the MFO metabolic pathways. Enzyme activities were determined by measuring the conversion of  $^{36}\text{Cl}$ -labeled MC to water-soluble products. Metabolic constants were then compared across species (mouse, rat, hamster and human). In the liver, the MFO activity was highest in the hamster, followed by the mouse, human and rat. Human values were much more variable than those of the rodent species. Human  $V_{max}$  for the liver MFO pathway ranged approximately an order of magnitude and human  $K_m$  varied approximately three-fold. GST activity in the liver was determined for mouse and human tissues only. Mouse liver had approximately 18-fold greater activity ( $V_{max}$ ) than human liver, but the human tissue had about a three-fold greater affinity constant ( $K_m$ ) for MC than the mouse.

In the lung, the activity of the MFO and GST enzymes was determined for a single substrate concentration. For the MFO pathway, mouse tissue had the highest activity, followed by hamster and rat. No MFO activity specific for MC was detected in the human lung tissue, although other MFO isozymes were demonstrated to be active in the tissue. For the GST pathway in lung, mouse tissue was the most active, followed by rat and human. No GST activity was detected in the hamster lung.

In humans, the MFO pathway has been measured *in vivo* as well as *in vitro* [Ex. 7-225]. Human *in vivo* experimentation was conducted by several investigators. Metabolism via the MFO pathway is relatively easy to measure because the end product is carbon monoxide [Ex. 7-24]. The metabolic rates measured *in vitro* were not similar to those measured *in vivo* after exposure to known concentrations

of MC, which means that *in vitro* measurements in human tissue (in particular for the GST pathway for which there are no human *in vivo* data) could not be used directly as a measure of metabolism. Human *in vivo* and *in vitro* MFO metabolism data were important in developing the pharmacokinetic models because they provided human data for MC-specific metabolism which could be used to help validate the models. Unfortunately, the modeling of the putative critical pathway for carcinogenesis (the GST pathway) could not be validated for humans. This is a weakness in the PBPK modeling for MC shared by all of the models, including OSHA's final PBPK analysis.

In the PBPK models submitted to OSHA, the human rate of metabolism of MC, particularly via the GST pathway, was based on data gathered from four liver samples and one pooled lung sample. Although the liver metabolic data were of the same magnitude as those collected by Green et al., Green's data were not considered in Reitz's model and the variability of those data was not assessed. Therefore, the estimates of the dose surrogates in Reitz's model were based on the average of four liver samples. Four liver samples are not nearly enough data to confidently estimate and account for human variability. Considerations of the variability and uncertainty of these data

are discussed in more detail later in this document.

The human lung data were even more limited. Four human lung samples were pooled to provide a single data point. This lack of lung tissue data is particularly critical in PBPK modeling when calculating the ratios of A1 and A2 (the distribution of metabolism between liver and lung tissue in humans). Errors in calculating these ratios will significantly affect the final risk estimates, as discussed by Mr. Harvey Clewell for the U.S. Navy [Ex. 96].

HSIA submitted additional data on the human metabolism of MC in the form of a study of GST metabolism in human liver samples conducted by Bogaards et al. [Ex. 127-16]. The human GST liver metabolism data collected in this study were not directly comparable to the data collected by Reitz or Green, because the Bogaards data were measured using a colorimetric method which was not as sensitive as the  $^{36}\text{Cl}$  method. Under contract to OSHA, Dr. Andrew Smith and Dr. Frederic Bois compared the data from different laboratories and collected under different methodologies and developed a correction factor across methodologies so that they could use all of the human metabolic data available in OSHA's final PBPK model [Ex. 128]. There are now over 30 data points for human liver *in vitro* metabolism by the GST pathway and 5 human lung data points (the additional lung data points were

reported in Green et al., Ex. 124A). OSHA determined that it was important to use as much of the available human data in its PBPK model for MC as scientifically justifiable. These data were used to estimate the variability and uncertainty surrounding the measures of human GST metabolism. Although the methodologies differed across studies, OSHA has adjusted and incorporated all of the available human data in its PBPK model.

(2) *Parallelogram approach.* When the metabolic rates for the MFO pathway measured *in vivo* and *in vitro* within each species were compared, it was determined that those rates were not equivalent. This meant that, unlike the case for some other chemical compounds, the *in vitro* GST data could not substitute directly for an *in vivo* measurement of metabolism. Reitz and Andersen [Ex. 7-225] suggested a "parallelogram" approach to the problem of non-comparability of *in vitro* and *in vivo* rates. This approach makes the assumption that the ratio of *in vivo* to *in vitro* measurements is roughly comparable across species (including humans). They measured metabolic rates of both pathways *in vitro* and *in vivo* in rodents and then used the average ratio of the *in vitro* to *in vivo* metabolic rate in three rodent species to extrapolate from *in vitro* rates in humans [Ex. 7-225] to an estimated *in vivo* value.

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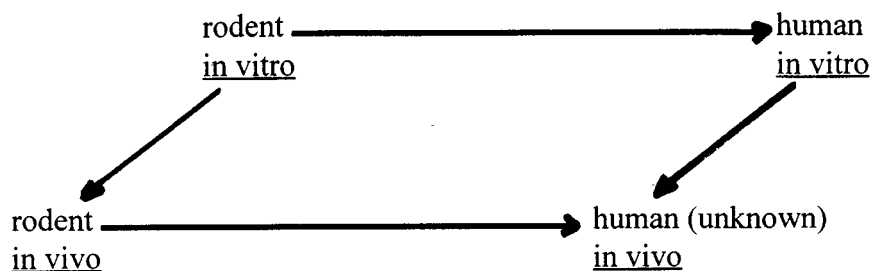


Figure VI-1: Schematic diagram of the parallelogram approach.

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$$\text{Assumption: } \frac{\text{rodent (in vitro)}}{\text{rodent (in vivo)}} = \frac{\text{human (in vitro)}}{\text{human (in vivo)}}$$

$$\text{or, human (in vivo)} = \frac{\text{rodent (in vivo)} \times \text{human (in vitro)}}{\text{rodent (in vitro)}}$$

Ron Brown [Ex. 25-E], an expert witness for OSHA, was concerned that "...the methodology used to extrapolate the *in vitro* data to the *in vivo* state is problematic and the accuracy of the human *in vitro* measurement of GST activity toward MC is uncertain." This may be due to the small sample size, variability in the laboratory analysis or inadequacy of the *in vitro* model. OSHA believes that this is a critical point of uncertainty in using the PBPK model for risk assessment. The Agency also notes that in the risk assessments using PBPK models submitted during the MC rulemaking, none used the parallelogram approach as the basis of determining human *in vivo* metabolic rates. Instead, allometric scaling was used to estimate human values. OSHA has conducted risk assessments using both the allometric approach (OSHA's final risk estimates) and the parallelogram approach (OSHA's alternative analysis). The Agency did this in order to determine what the risk estimates would be if all possible quantitative data were used to the fullest extent, regardless of the uncertainties in the data.

OSHA agrees that evidence presented in the record generally supports the GST pathway as a plausible carcinogenic mechanism of action of MC. The Agency remains concerned, however, that sole reliance on the GST pathway may show insufficient consideration for potential contributions of the parent compound and/or metabolites of the MFO pathway to the carcinogenesis of MC. It is clear that ambient MC concentration is dose-related to tumor response. It has not been shown with any certainty that MC GST metabolites are related to tumor response across species. Thus, there is greater confidence that the lifetime bioassays predict MC carcinogenicity in humans than there is that cancer occurred through a specific mechanism, and even less confidence that the metabolic rates measured *in vitro* accurately measure differences in species that correlate to tumor development. This is particularly true for lung metabolism where only one pooled and five individual human samples were analyzed. Notwithstanding the uncertainties described above, the Agency believes that the hypothesis that GST is the carcinogenic pathway presents a plausible mechanism of action for MC and is sufficiently well-developed to warrant the use of PBPK modeling of the GST pathway as the dose surrogate of choice in the quantitative risk assessment for MC.

*d. Structure of the MC PBPK model.* The PBPK models described below are

based on the model originally submitted by Dr. Reitz on behalf of HSIA in 1992 [Ex. 7-225]. Over the years since the first submission of a MC PBPK model to OSHA, significant improvements have been made in model structure and in the data collected for PBPK modeling, especially in how the uncertainty and variability in the data are treated. The general structure of the models submitted to OSHA are described below, followed by a description of the parameters used in the various models. Next follows a description of how the variability, uncertainty, and sensitivity of the models to uncertainty have been assessed, noting the improvements that have been made in developing methods to handle these issues. This is followed by a comparison of the risk estimates generated by these models. Finally, OSHA's final risk assessment is described. This risk assessment incorporates lessons learned from previous models and uses all of the available, appropriate, quantifiable data in a Bayesian approach to modeling the dose metric for MC.

In the PBPK model submitted by Dr. Reitz of HSIA [Ex. 7-225], a series of differential equations was used to model the mass balance of MC and its metabolites in five physiologically-defined compartments, including the lung, liver, richly perfused tissue, slowly perfused tissue, and fat. Metabolism via the MFO pathway was described by saturable Michaelis-Menten kinetic equations and GST metabolism was modeled using first-order nonsaturable kinetics. With the exception of the PBPK model submitted by ICI [Ex. 14A], all of the PBPK models submitted to the Agency followed these assumptions regarding the metabolism of MC. The rate constants for the metabolic equations were estimated based on measurements of the partition coefficients, allometric approximations of the physiological constants (e.g., lung weight), and estimations (i.e., allometric scaling of rodent data, estimations made using the parallelogram approach, etc.) of the biochemical constants (e.g., Michaelis-Menten constants).

NIOSH presented a PBPK model in 1993 [Ex. 94], also structurally based on the Reitz-Andersen model, but with modifications to the human breathing rate and cardiac output to account for uptake of MC in physically active workers, rather than at-rest humans or humans involved in light activity, and including an analysis of the variability of the human metabolic parameters. Specifically, NIOSH compared estimates derived from the arithmetic average of the human GST metabolism data with the individual human liver data points

to estimate the uncertainty in an individual's risk of cancer from occupational MC exposure. This approach began to incorporate some necessary features, such as a special focus on physically active workers and the variability of human metabolic parameters, but did not attempt to quantify the uncertainty and variability of the individual parameters and their contribution to the uncertainty associated with the PBPK model.

Mr. Harvey Clewell, representing the U.S. Navy, also submitted several PBPK models to OSHA. In his initial submission (1992), Mr. Clewell modified an existing PBPK model [Ex. 7-125] to include more recent data on the mouse blood/air partition coefficient [Ex. 19-59]. In a second PBPK model, he "started from scratch" to construct a model based on data derived from sources independent of the previous work of Reitz and Andersen [Ex. 23-14], which was described in Mr. Clewell's testimony [Tr. 2361, 10/15/92]. This model was structurally similar to the model presented by HSIA with the following exceptions: it featured three lumped compartments (slowly perfused, moderately perfused and rapidly perfused) based on tissue kinetic constants rather than the earlier two lumped compartment models based on tissue blood volumes; and the mouse blood/air partition coefficient was corrected to 19.4 instead of the earlier 8.29 on the basis of more recent data. A third model submitted by Mr. Clewell was identical in structure to the Reitz/Andersen model, but incorporated the more recent experimental data on the partition coefficients and the more recent mouse metabolism data [Ex. 96]. OSHA used Mr. Clewell's third model in its comparison of PBPK-derived risk estimates because of its similarity in structure to the original Reitz model and its incorporation of the most recent experimental data.

In his third model, Clewell either derived probability distributions for each parameter from the literature or estimated distributions for those parameters for which data were not available, and conducted Monte Carlo simulations to derive output distributions for the dose surrogates. These distributions of dose surrogates were then used to derive four risk estimates: the doses input into the multistage dose-response analysis of the tumor bioassay were derived either from the mean or from the 95th percentile of the output distribution of PBPK parameters, and these in turn were coupled with the either the MLE or the UCL of the distribution of possible values of the multistage model

parameters. This analysis was an advance over that of previous models because it took into account some of the uncertainty and variability known to be associated with the data used in the PBPK model.

After evaluating these submitted models, OSHA determined that Clewell's model provided the best prototype on which to base its final PBPK modeling approach for MC. Therefore, the Agency worked with Drs. Smith and Bois to review Clewell's model and with the assistance of Dr. Hattis, to develop a refined PBPK modeling approach with a more

sophisticated analysis of variability and uncertainty (and other refinements as described below). In this way the Agency developed an approach which would incorporate what was learned in the development of earlier PBPK models and make use of as much of the available physiological and metabolic data in the record as possible. Clewell's model was chosen for comparison, because this was the only model to provide a systematic analysis of the uncertainty, variability and sensitivity of the model using Monte Carlo techniques. OSHA's final risk

assessment approach is described in greater detail below.

*e. Choice of parameters for PBPK modeling.* The definitions of the parameters used in the models described above are contained in Table VI-2. Note that not all parameters were used in each model and slightly different variable names were used by different investigators. For example, OSHA's final analysis contains a bone marrow compartment, while Clewell's model did not. OSHA refers to the blood flow for poorly (or slowly) perfused tissues as "QppC," while Clewell used "QSC."

TABLE VI-2.—DEFINITIONS OF PHARMACOKINETIC PARAMETERS

| Parameter (units)                               | Definition  |
|---|---|
| BW (kg) .....                                   | Body weight in kg. Human body weights were assumed to be 70-kg (Reference Man). Mouse body weights were the average weight of mice in the NTP bioassay.   |
| QPC unscaled (1/hr, 1 kg BW) .....              | Breathing rate. $QPC = QP(1/hr)/BW^{.75}$ where $QP$ = alveolar ventilation rate. Human $QP$ was based on rate of 9.6 m <sup>3</sup> /8-hr (converted 1/hr and adjusted to alveolar ventilation (= 0.70 total ventilation) except in NIOSH and OSHA-modified models. Mouse $QP$ = (24.3 1/hr)(0.70 alveolar/total). |
| QCC unscaled (1/hr, 1 kg BW) .....              | Cardiac output. $QCC = QC(1/hr)/BW^{.75}$ where $QC$ = cardiac output in 1/hr. Reitz set $QC = QP$ . Clewell and NIOSH based human $QC$ on Astrand et al. [Ex. 7-120] data on cardiac output and breathing rate vs. workload.   |
| VPR (ratio, unitless) .....                     | Alveolar ventilation/perfusion ratio.   |
| <b>Blood flows to tissues</b>                   |   |
| QGC or QgiC (fraction of cardiac output) .....  | Blood flow to gastrointestinal tract as a fraction of cardiac output. $QGC = QG/QC$ .   |
| QLC or QliC (fraction of cardiac output) .....  | Blood flow to liver as a fraction of cardiac output. $QLC = QL/QC$ .  |
| QFC or QfatC (fraction of cardiac output) ..... | Blood flow to fat as a fraction of cardiac output. $QFC = QF/QC$ .  |
| QSC or QppC (fraction of cardiac output) .....  | Blood flow to slowly (or poorly) perfused tissues as a fraction of cardiac output. $QSC = QS/QC$ .  |
| QRC or QwpC (fraction of cardiac output) .....  | Blood flow to rapidly (or well) perfused tissues as a fraction of cardiac output. $QRC = QR/QC$ .   |
| QmarC (fraction of cardiac output) .....        | Blood flow to bone marrow as a fraction of cardiac output.  |
| <b>Tissue volumes</b>                           |   |
| VGC or VgiC (fraction of body weight) .....     | Volume of GI tract as a fraction of body weight. $VGC = VG/BW$ .  |
| VLC or VliC (fraction of body weight) .....     | Volume of liver as a fraction of body weight. $VLC = VL/BW$ .   |
| VFC or VfatC (fraction of body weight) .....    | Volume of fat as a fraction of body weight. $VFC = VF/BW$ .   |
| VSC or VppC (fraction of body weight) .....     | Volume of slowly (or poorly) perfused tissues as a fraction of body weight. $VSC = VS/BW$ .   |
| VRC or VwpP (fraction of body weight) .....     | Volume of rapidly (or well) perfused tissues as a fraction of body weight. $VRC = VR/BW$ .  |
| VluC (fraction of body weight) .....            | Volume of lung as a fraction of body weight.  |
| VmarC (fraction of body weight) .....           | Volume of bone marrow as a fraction of body weight.   |
| <b>Partition coefficients</b>                   |   |
| PB or Pblo .....                                | Blood/air partition coefficient.  |
| PG or Pgi .....                                 | GI tract/blood partition coefficient (GI tract/air divided by PB).  |
| PL or Pli .....                                 | Liver/blood partition coefficient (Liver/air divided by PB).  |
| PF or Pfat .....                                | Fat/blood partition coefficient (Fat/air divided by PB).  |
| PS or Ppp .....                                 | Slowly (or poorly) perfused tissue/blood partition coefficient (Slowly perfused tissue/air divided by PB).  |
| PR or Pwp .....                                 | Rapidly (or well) perfused tissue/blood partition coefficient (Rapidly perfused tissue/air divided by PB).  |
| PLU or Plu .....                                | Lung/blood partition coefficient (Lung/air divided by PB).  |
| Pmar .....                                      | Bone marrow:air partition coefficient.  |
| <b>Metabolic parameters</b>                     |   |
| VMAXC unscaled (mg/hr, 1 kg animal) .....       | MFO pathway Michaelis-Menten maximum velocity for MC metabolism. $VMAXC = VMAX$ (mg/hr)/ $BW^{.75}$ .   |
| KM (mg/l) .....                                 | MFO pathway Michaelis-Menten affinity constant for MC metabolism.   |

TABLE VI-2.—DEFINITIONS OF PHARMACOKINETIC PARAMETERS—Continued

| Parameter (units)                       | Definition   |
|---|--|
| KFC, unscaled, (/hr, 1 kg animal) ..... | GST pathway 1st order kinetic rate constant for MC metabolism. $KFC = KF / (hr)(BW^{.25})$ .                 |
| A1 (ratio) .....                        | Ratio of distribution of MFO pathway MC metabolism between lung and liver. $A1 = VMAXC(lung)/VMAXC(liver)$ . |
| A2 (ratio) .....                        | Ratio of distribution of GST pathway MC metabolism between lung and liver. $A2 = KFC(lung)/KFC(liver)$ .     |
| B1 (ratio) .....                        | Ratio of lung and liver tissue content of microsomal protein.  |
| B2 (ratio) .....                        | Ratio of lung and liver tissue content of cytosolic protein.   |
| Sp—Kf .....                             | Allometric scaling power for body weight scaling of KFC from mice to humans.                                 |

The MC physiologically-based pharmacokinetic (PBPK) models discussed here contain the following types of parameters as defined above: body weight, breathing rate, cardiac output, blood flows to tissue compartments (as a fraction of the cardiac output), volumes of tissue compartments (as a fraction of body weight), partition coefficients, the metabolic parameters (the Michaelis-Menten parameters, Vmax and Km, for the MFO pathway and the 1st-order rate constant, Kf, for the GST pathway) and the ratio of the pathway-specific metabolic capacity between the major

metabolic sites (lung and liver). Differences in model structure (such as choice of lumped tissue compartments) and differences in sources of data for individual parameters lead to differences in the parameter values used in different models.

The parameter values (point estimates) used in the PBPK models reviewed by OSHA are presented in Table VI-3. The parameter distributions used by OSHA in its analysis are presented later.

As far as OSHA could determine, the parameters chosen by HSIA were those presented in Reitz's 1989 paper [Ex. 21–

53] except that OSHA's preferred values for breathing rates (based on 9.6 m<sup>3</sup>/workday) and 8-hour human exposures were used. The model submitted by NIOSH used the parameters and computer code from the Reitz model, except for the human breathing rate, human cardiac output and human metabolic parameters. The parameters used by Clewell were summarized in his post-hearing submission [Ex. 96], which included more recent experimental data for the partition coefficients and mouse metabolic parameters and a different scaling for human cardiac output.

TABLE VI-3.—PARAMETERS USED IN PBPK MODELS REVIEWED BY OSHA

| Model<br>Parameter   | Clewell [Ex. 96] |       | NIOSH [Ex. 23–18] |                              | HSIA [Ex. 19–45] |        |
|--|------------------|-------|-------------------|------------------------------|------------------|--------|
|  | Mouse            | Human | Mouse             | Human                        | Mouse            | Human  |
| BW (kg) .....  | 0.0345           | 70    | 0.0345            | 70                           | 0.0345           | 70     |
| QPC, unscaled alveolar ventilation (1/hr, 1 kg animal) .....                           | 29.0             | 35    | 29.0              | 43.1                         | 29.0             | 35.0   |
| QCC, unscaled cardiac output (1/hr, 1 kg animal) .....                                 | 16.5             | 18    | 29.0              | 20.9                         | 29.0             | 35.0   |
| QGC <sup>a</sup> , flow to GI tract (fraction of cardiac output) .....                 | 0.165            | 0.195 | 0.0               | 0.0                          | 0.0              | 0.0    |
| QLC <sup>a</sup> , flow to liver (fraction of cardiac output) .....                    | 0.035            | 0.07  | 0.24              | 0.2093                       | 0.24             | 0.24   |
| QFC <sup>a</sup> , flow to fat (fraction of cardiac output) .....                      | 0.03             | 0.05  | 0.05              | 0.040                        | 0.05             | 0.05   |
| QSC <sup>a</sup> , flow to slowly perfused tissues (fraction of cardiac output) .....  | 0.25             | 0.24  | 0.19              | 0.4319                       | 0.19             | 0.19   |
| QRC <sup>a</sup> , flow to rapidly perfused tissues (fraction of cardiac output) ..... | 0.52             | 0.445 | 0.52              | 0.3188                       | 0.52             | 0.52   |
| VGC, GI volume (fraction of BW) .....  | 0.031            | 0.045 | 0.0               | 0.0                          | 0.0              | 0.0    |
| VLC, liver volume (fraction of BW) .....   | 0.046            | 0.023 | 0.04              | 0.0314                       | 0.04             | 0.0314 |
| VFC, fat volume (fraction of BW) .....   | 0.100            | 0.16  | 0.07              | 0.231                        | 0.07             | 0.231  |
| VSC, slowly perfused tissue volume (fraction of BW) .....                              | 0.513            | 0.48  | 0.75              | 0.621                        | 0.75             | 0.621  |
| VRG, rapidly perfused tissue volume (fraction of BW) .....                             | 0.041            | 0.033 | 0.05              | 0.0371                       | 0.05             | 0.0371 |
| VLUC, lung volume (fraction of BW) .....   | 0.008            | 0.006 | 0.012             | 0.011                        | 0.012            | 0.011  |
| PB, blood/air part. coeff .....  | 23.0             | 12.9  | 8.29              | 9.7                          | 8.29             | 9.7    |
| PG, GI tract/air part. coeff .....   | 0.52             | 0.93  | NA                | NA                           | NA               | NA     |
| PL, liver/blood part. coeff .....  | 1.6              | 2.9   | 1.71              | 1.46                         | 1.71             | 1.46   |
| PF, fat/blood part. coeff .....  | 5.1              | 9.1   | 14.5              | 12.4                         | 14.5             | 12.4   |
| PS, slowly perf./blood part. coeff .....   | 0.44             | 0.78  | 0.96              | 0.82                         | 0.96             | 0.82   |
| PR, rapidly perf./blood part. coeff .....  | 0.52             | 0.93  | 1.71              | 1.46                         | 1.71             | 1.46   |
| PLU, lung/blood part. coeff .....  | 0.46             | 0.82  | 1.71              | 1.46                         | 1.71             | 1.46   |
| VMAXC mg/hr, 1 kg animal (unscaled) .....  | 13.4             | 5.0   | 13.2              | 3.98<br>1.15<br>9.81<br>4.71 | 13.2             | 4.9    |
| KM (mg/L) .....  | 1.35             | 0.4   | 0.396             | 0.72<br>0.55<br>0.26<br>0.79 | 0.396            | 0.580  |



TABLE VI-3.—PARAMETERS USED IN PBPK MODELS REVIEWED BY OSHA—Continued

| Model                                 | Clewell [Ex. 96] |       | NIOSH [Ex. 23-18] |                              | HSIA [Ex. 19-45] |         |
|---------------------------------------|------------------|-------|-------------------|------------------------------|------------------|---------|
| Parameter                             | Mouse            | Human | Mouse             | Human                        | Mouse            | Human   |
| KFC /hr, 1 kg animal (unscaled) ..... | 1.5              | 1.5   | 1.73              | 1.56<br>0.00<br>1.62<br>1.79 | 1.73             | 1.24    |
| A1 (Vmaxc(lung)/Vmaxc(liver)) .....   | 0.41             | 0.015 | 0.416             | 0.00143                      | 0.416            | 0.00143 |
| A2 (KFC(lung)/KFC(liver)) .....       | 0.28             | 0.18  | 0.137             | 0.18                         | 0.137            | 0.18    |

<sup>a</sup>QGC + QLC + QFC + QSC + QRC *MUST* = 1.00.

*f. Assessment of the sensitivity and uncertainty of the PBPK model.* In the NPRM, OSHA expressed concern that, if PBPK models were used to adjust risk assessments, the uncertainty in PBPK modeling should be adequately addressed. Specifically, OSHA was concerned that the uncertainty in the mechanism of action and the lack of human lung metabolism data were the greatest obstacles to incorporation of pharmacokinetic data into the MC final risk assessment. Many of the uncertainties in model parameters have been quantified by various hearing participants and are summarized below. The quantification of these uncertainties, however, did not address OSHA's primary concerns regarding the mechanism of action and the distribution of metabolism between lung and liver. OSHA's analyses of the uncertainty and variability of parameters in the PBPK model are presented with its risk assessment later in this document.

The concepts of uncertainty, variability and sensitivity in PBPK modeling were defined in comments submitted by the U.S. Navy [Ex. 19-59]:

As it relates to the issue of using PBPK modeling in risk assessment, uncertainty can be defined as the possible error in estimating the "true" value of a parameter for a representative ("average") animal. Variability, on the other hand, should only be considered to represent true interindividual differences.

The normalized sensitivity coefficient gives the percentage change in a model output due to a percentage change in the parameter value and represents the relative importance of the parameter to the model output under the conditions of the simulation.

Each of these quantities is of concern for risk assessment and PBPK modeling. For example, we know that there is variability or inter-individual heterogeneity in the body weights of humans (and mice), yet we estimate risks for an average member of the population (70 kg in humans, average bioassay weight in mice). For many parameters, the interindividual

variability may not be known and must be estimated.

Uncertainty in estimation of the value of a parameter representing an average member of a population is primarily due to laboratory measurement and related errors. Measurement errors, in many cases, can be quantified or estimated so that the potential impact of this uncertainty on the outcome of the PBPK modeling can be assessed.

The sensitivity of the model to particular parameters is useful for determining which experiments should be conducted to confirm parameters and to determine the amount of confidence that PBPK model outputs merit. For example, when a sensitivity analysis is conducted and it is determined that the model outcomes are not very sensitive to changes in the definitions of the lumped tissue volumes, it suggests that there is little need to conduct experiments to describe those relationships more precisely. Similarly, even though the lumped tissue volume does not represent a "true" biological quantity, there is confidence that its precise definition is not critically important in PBPK model outcomes. Therefore, if the only large (quantifiable) uncertainty resides in this measurement, one would have greater confidence that the model predictions were reasonably accurate. Therefore, it is instructive to understand which parameters influence the model outcomes to the greatest degree. Conversely, if the PBPK model outputs are sensitive to a parameter which has not been precisely described (such as the distribution of GST metabolism between lung and liver), the confidence in model outputs is correspondingly reduced.

Various investigators have attempted to determine the sensitivity of the PBPK models to parameter values and to characterize the uncertainty and variability within parameters in the models. The first attempt to describe the sensitivity of the Reitz's original PBPK model was performed by the Consumer Product Safety Commission (CPSC).

The CPSC conducted a sensitivity analysis of the metabolic parameters, Km, Vmax and Kf, in the "Updated Risk Assessment for Methylene Chloride" [Ex. 7-126]. They analyzed the sensitivity of the model by selecting alternative point estimates for the metabolic parameters and determining what the resulting ratio of GST metabolite at 4000 ppm vs. 1 ppm would be. This analysis shows how this ratio would vary if the metabolic parameters used in the model were higher or lower than the measured values as selected by CPSC. The results showed that the ratio of the GST metabolite in the liver at 4000 ppm to the GST metabolite at 1 ppm (or the ratio of the GST metabolite in the lung at 4000 ppm to the GST metabolite at 1 ppm) was relatively insensitive to the value of Kf (when CPSC varied Kf from 0.01 to 5.3, while Km and Vmax were held constant at Reitz-Andersen values).

HSIA presented a sensitivity analysis of the PBPK parameters from the Reitz (HSIA) model in the testimony of Dr. Reitz [Ex. 23-21A]. Results were presented for mice at 4000 ppm, mice at 1 ppm, humans at 1000 ppm and humans at 1 ppm. In the first analysis (mice at 4000 ppm), the most sensitive parameters were determined to be PB (blood:air partition coefficient) and Kf (metabolic parameter for the GST pathway). The authors observed that at high MC exposure levels the model output was at least an order of magnitude less sensitive to changes in the other sixteen parameters investigated.

When mice were exposed to lower concentrations of MC (1 ppm) Vmax and Km for the MFO pathway were the most sensitive parameters (sensitivity coefficient was over 120% for each of these parameters). In addition, several other parameters were found to exert a significant influence on model outputs: QP, QL, PB, VLu, and KF.

In humans, at high concentrations (> 1000 ppm) the results were similar to those observed in mice: the model was most sensitive to PB and KF, with

sensitivity coefficients of 87% and 97%, respectively. In addition, the human model was also sensitive to the value chosen for the QP (sensitivity coefficient = 43%).

In humans, at 1 ppm MC, Km and Vmax for the MFO pathway were the most sensitive parameters out of the six parameters which had a significant effect upon model outputs: QP, QL, PB, Vmax, Km, and KF.

This type of sensitivity analysis improves on that conducted by the CPSC, because it looks at more of the parameters. It is still deficient, however, because it examines the effect of each parameter individually, and because it does not examine the effect of uncertainty in two key parameters, A1 and A2 (the ratios of distribution of the MFO and GST pathways between lung and liver), on the outcomes of the modeling.

Mr. Clewell [Ex. 19–59] also conducted a sensitivity analysis to determine the impact of uncertainty in PBPK parameters on the model outcomes. In contrast to the HSIA analysis, he examined the sensitivity of the outcomes to the ratios A1 and A2, and he chose a more realistic occupational exposure level (100 ppm). He found that for mice at 4000 ppm, the most sensitive parameters for estimation of lung tumors were KF, A2, and PB. In the liver, the most sensitive parameters were KF and PB, which agrees with the results of the HSIA analysis. For humans at 100 ppm, the most sensitive parameters for estimating lung tumors were KF and A2. Other parameters with significant effects on model outcomes were PB, QPC, BW, KM, QCC, and QLC. The most sensitive parameters for estimating liver tumors were VMAX, KF, QPC and BW, while PB, KM, QCC and QLC also produced significant effects on model outcomes.

In all of these analyses, the PBPK models were clearly sensitive to the values chosen for the metabolic parameters, especially the GST metabolic parameter (KF). Other parameters with consistently significant impact on the outcomes of the model included breathing rate (QP) and distribution of GST metabolism between lung and liver (A2). These analyses suggest that additional studies to quantify the metabolic parameters (KF, KM and VMAX), breathing rates (QP) and distribution of GST metabolism between lung and liver (A2) would increase confidence in the model outcomes. Characterization of the distribution of metabolism between lung and liver is particularly critical because estimates for human lung metabolism were initially based on one pooled

sample of lung tissue, and the variability and uncertainty of the value of this parameter has not been quantified.

Some analysts [Ex. 21–52] have suggested that the uncertainty is increased in risk assessments based on PBPK as compared to applied-dose risk assessments, because some methods of quantifying the uncertainty result in rather broad distributions of uncertainties. OSHA, in contrast, agrees with most commenters that quantifying uncertainty in a PBPK model or risk assessment does not increase the uncertainty. The Agency stresses that the appearance of increasing uncertainty with the identification of sources of uncertainty almost certainly means that the original uncertainty was underestimated. (In fact, since many assessors have not attempted even to quantify the uncertainty in applied-dose risk assessments, the uncertainty has often been infinitely underestimated.) When conducting a risk assessment using PBPK that appears to increase the uncertainty over delivered-dose methodologies, the investigator should go back and recalibrate what the uncertainty in the original analysis likely was, in light of the sources of uncertainty identified using PBPK. This would tend to broaden the confidence limits of the traditional risk assessments, almost certainly beyond the limits generated in a thoughtful PBPK-based assessment. For example, many analyses using delivered dose assume that in the interspecies scaling factor,  $BW^x$ ,  $x$  is known with perfect certainty (e.g., it is known to equal 2/3 or 1.0). An analysis that uses an empirically-derived probability distribution for  $x$ , which might reasonably extend from approximately 0.6 to approximately 1.0, would yield a rather broad distribution of uncertainty in the resulting estimate of risk.

The Agency also agrees that the primary uncertainties lie in the choice of the dose surrogate and assumptions regarding cross-species scaling. Clewell [Ex. 23–14] investigated the uncertainty of the PBPK parameters using Monte Carlo analyses of the assumed distributions of uncertainty of each parameter. The resulting estimates of dose surrogate values were characterized by a mean of the distribution and an upper 95th percentile estimate. Mr. Clewell stated [Ex. 19–59]:

[T]he use of the 95th percentile of the distribution of estimates accounts for additional uncertainty concerning the true values of the PBPK parameters for the bioassay animals and humans.

Mr. Clewell recommended that OSHA use the upper 95th percentile of the Monte Carlo distribution of GST metabolites (from PBPK modeling) as an input to the multistage model to generate risk estimates, and then use of the MLE from the multistage model in those risk estimates, in accordance with previous OSHA risk assessments. He remarked that use of the upper 95th percentile of the PBPK output would be a reasonable mechanism to account for the uncertainty quantified in these analyses. Using the upper 95th percentile of the distribution of GST metabolites, Mr. Clewell's risk estimate for lifetime occupational exposure to 25 ppm MC was 0.9 deaths per 1000 using the MLE of the multistage model, and 1.1 per 1000 using the 95th percentile upper confidence limit (UCL) from the multistage model. Using the mean of the distribution of GST metabolites, his MLE risk estimate was 0.28 deaths per 1000 at the same exposure level, with an UCL of 0.35/1000.

The HSIA disagreed with using the upper 95th percentile for estimating risks, and stated [Ex. 105]:

[T]he analyses conducted by Clewell et al. indicate that consideration of model parameter variability does not contribute orders of magnitude to the uncertainty associated with PB–PK risk assessments. Further, the uncertainty associated with PB–PK risk assessments is significantly less than that associated with risk assessments that fail to consider pharmacokinetics. The uncertainty in PB–PK based procedures is simply more readily available for calculation.

OSHA disagrees with the HSIA that the uncertainty and variability associated with PBPK risk assessments is significantly less than that associated with risk assessments that fail to consider pharmacokinetics. Quantification of uncertainty does not equate with reducing uncertainty in an analysis. In fact, at a different level, the assumptions made regarding mechanism of action of MC and extrapolation of lung metabolic rates from one human *in vitro* sample may serve to underestimate the uncertainty inherent in the PBPK-based risk assessment if the underlying assumptions are wrong. Also, as stated above, identification of uncertainty may lead us to recalibrate the uncertainty associated with traditional risk assessment methods. In any event, the possibility that using PBPK significantly reduces uncertainty does not affect the need to account for whatever uncertainty remains.

In addition, OSHA agrees with Clewell that using the upper 95th percentile of the Monte Carlo distribution of GST metabolites as input

to the multistage model is a reasonable way to incorporate the quantifiable uncertainty and variability into a risk assessment. In its final risk estimates, OSHA has used the upper 95th percentile on the distribution of GST metabolites from the Bayesian analysis as the input to the multistage model, as described later in this document.

#### *E. Other Risk Estimates Based on PBPK Models Prior to OSHA's Final Analysis.*

A PBPK model can produce estimates of target tissue doses (or dose surrogates) for different hypotheses of action of a chemical. The appropriate choice of target tissue dose can greatly influence risk estimates based on that dose. For MC, the dose surrogate that has been used most frequently to estimate cancer risks is the amount of GST metabolite produced. The amount of GST metabolite can then be used to extrapolate from a high bioassay dose of MC to a low occupational (or environmental) dose of MC and from mouse MC metabolic rates to human metabolic rates.

In the NPRM, OSHA reviewed available risk assessments for MC that used PBPK modeling in a variety of ways. The Food and Drug Administration risk assessment [Ex. 6-1] was not adjusted to account for pharmacokinetic information. The Consumer Product Safety Commission, in its "Updated risk assessment for methylene chloride" [Ex. 7-126], used pharmacokinetic data to adjust for differences in metabolism in extrapolating from high dose (4000 ppm mouse bioassay) to low dose (1 ppm) exposures, but did not adjust for interspecies differences in the metabolism of MC. The resulting risk estimate was approximately 2-fold

lower than a risk estimate using applied dose.

The U.S. EPA analyzed the MC pharmacokinetic data in its documents, "Technical analysis of new methods and data regarding dichloromethane hazard assessment" [Ex. 7-129] and "Update to the Health Assessment Document and Addendum for dichloromethane (methylene chloride): pharmacokinetics, mechanism of action, and epidemiology" [Ex. 7-128]. The EPA used the PBPK data to adjust its risk estimates in its Integrated Risk Information System (IRIS) database. Adjustments were made for high-to-low dose and cross-species extrapolation. EPA's risk estimates for low human exposures to MC were decreased by approximately a factor of 9 from its risk estimates made without consideration of PBPK data.

The HSIA [Ex. 105] and ECETOC [Ex. 14] also submitted risk assessments based on PBPK data. The primary difference between the HSIA and the EPA risk estimates was that the HSIA did not use a surface area correction to account for interspecies differences other than pharmacokinetics (e.g., pharmacodynamic differences) while the EPA did. Also, HSIA's risk estimates used OSHA's preferred breathing rates and an occupational exposure scenario. ECETOC based its risk estimates on different measures of human MC metabolism. In a pre-hearing submission, "Using PB-PK Models for Risk Assessment with Methylene Chloride (Comparison of U.S. and U.K. procedures)" [Ex. 19-83A], scientists from the U.S. and the U.K. compared methodologies for using PBPK data in the MC risk assessment and presented a consensus opinion that OSHA should use the methodology developed by Dr.

Richard Reitz [Ex. 7-225] for the U.S. For this reason, OSHA evaluated Dr. Reitz's analysis, as presented by the HSIA, and did not separately consider the ECETOC risk assessment.

As described previously, Clewell [Ex. 96] and NIOSH [Ex. 94] have submitted analyses of the PBPK data and risk assessments based on those analyses. Both of these analyses used PBPK modeling of the amount of GST metabolites produced in their estimates of carcinogenic risks.

OSHA has evaluated the data in the rulemaking record and has concluded that, if PBPK modeling is used to adjust estimates of risk, the weight of evidence supports using the amount of GST metabolites as the preferred surrogate for target tissue dose. The amount of GST metabolites predicted by the PBPK model varies depending upon the values or distributions chosen for the parameters in the model.

Of the risk assessments described above, OSHA has chosen to compare risks estimated using PBPK models submitted by Reitz et al., Clewell et al. and NIOSH with applied dose methodology using either of two scaling assumptions: the inhaled dose in mg/kg/day (the estimates of risk presented in the NPRM) and ppm-to-ppm extrapolation. OSHA evaluated the methodologies used in developing these risk estimates before developing its final risk estimates, which are presented in the next section.

The risk estimates derived from using PBPK with the multistage dose-response model submitted to the Agency by Reitz et al., Clewell et al., and NIOSH, and the risk estimates derived from applied dose methodologies, are shown in Table VI-4.

TABLE VI-4.—LIFETIME EXCESS RISK ESTIMATES (PER 1000) FROM OCCUPATIONAL EXPOSURE BASED ON FEMALE MOUSE LUNG TUMOR DATA

| Model   | MLE (UCL)**       |                   |             |
|---|-------------------|-------------------|-------------|
|   | 25 ppm            | 50 ppm            | 500 ppm     |
| OSHA NPRM Risk Assessment (mg/kg/d, BW extrapolation) without PBPK Adjustment ..... | 2.32 (2.97) ..... | 4.64 (5.92) ..... | 45.5 (57.7) |
| PPM to PPM extrapolation without PBPK Adjustment .....                              | 11.3 (14.4) ..... | 22.4 (28.5) ..... | 203 (251)   |
| PBPK Reitz female mouse lung—Reitz human (HSIA assumptions) .....                   | 0.43 (0.53) ..... | 0.93 (1.17) ..... | 14.3 (17.9) |
| PBPK Reitz female mouse lung—Dankovic average human (NIOSH assumptions) .....       | 0.81 (1.02) ..... | 1.69 (2.12) ..... | 15.0 (18.7) |
| PBPK Clewell female mouse lung—Clewell human (Navy assumptions)* .....              | 0.91 (1.14) ..... | 1.88 (2.36) ..... | 27.5 (34.2) |
| OSHA Final Risk Assessment (female mouse lung with PBPK) .....                      | 3.62 .....        | 7.47 .....        | 125.8       |

\* Upper 95th percentile of the GST metabolites distribution was used as input in the multistage model.

\*\* Maximum likelihood estimates and 95th percentile upper confidence limit (in parentheses) of the multistage dose-response function.

Of those risk estimates considered by OSHA prior to its final risk assessment, the risk estimates for lifetime occupational exposure to the 8-hour TWA PEL of 25 ppm ranged from 0.43

per 1000 to 11.3 per 1000. The risk assessment presented in the NPRM was based on a body weight extrapolation from mice to humans of a mg/kg/day dose of MC. Mr. Harvey Clewell [Ex. 19-

59] stated that this dose was not a useful dose for estimating risks from volatile solvents such as MC. He suggested that, if PBPK modeling was not used to estimate target tissue dose (his preferred

method of estimating risk), then a ppm-to-ppm extrapolation would be more appropriate. The ppm-to-ppm extrapolation resulted in an estimated risk of 11.3 deaths per 1000 after lifetime occupational exposure to 25 ppm. However, the ppm-to-ppm extrapolation is generally preferred for site-of-contact tumors. Although it is possible that the MC lung tumors were the result of a site-of-contact mechanism of action, the data are more supportive of a systemic, genotoxic mechanism mediated through metabolites of MC. In addition, the liver tumors are clearly not the result of a site-of-contact carcinogen because the liver is not a site of contact during inhalation bioassays.

Several commenters [Exs. 19–26, 19–28, 19–29, 19–45, 19–48, 19–57, 19–59, 25–E, 25–I] suggested using PBPK modeling to estimate target tissue dose and to account for differences in metabolism at high and low doses and differences in metabolism of MC across species. OSHA compared three sets of parameters in the PBPK models submitted by interested parties to adjust the dose across species and across doses. The risk estimates for those models (using the MLE of the multistage model parameters) ranged from 0.43 to 0.91 deaths per 1000 after lifetime occupational exposure to 25 ppm. Mr. Clewell's risk estimate (0.91/1000 MLE), unlike the other PBPK analyses, represent the upper 95th percentile of the Monte Carlo distribution of GST metabolites as input into the multistage model. The Monte Carlo simulation takes into account the assumed distribution of values for each parameter, including the parameters used to estimate human metabolism of MC. The other PBPK models used point estimates instead of distributions for the PBPK parameters, and therefore it is not known whether these are central estimates or upper bounds. OSHA agrees that the distributional approach used by Clewell is a reasonable way to account for the uncertainty and variability inherent in PBPK modeling, and that uncertainty and variability must be considered in any useful risk assessment. The Agency has used the upper 95th percentile on the distribution of GST metabolites from the Bayesian modeling, coupled with the MLEs of the multistage model parameters, for its final estimates of MC risk.

OSHA has concluded that all the risk estimates presented above support an 8-hour TWA PEL of 25 ppm or lower. The risks estimated from the PBPK models were less than an order of magnitude different from estimates of risk based on applied dose methodology. Either with

or without PBPK modeling, the estimates of risk at 25 ppm clearly indicate a significant risk.

The risks estimated from these PBPK models and ppm-to-ppm extrapolation offer a range of risks which might be expected after lifetime occupational exposure to MC. OSHA has assessed these models and has decided to modify and expand on the submitted PBPK and uncertainty analyses in its final estimates of cancer risk, in order to give full consideration to all of the available data. This analysis is presented in the next section.

#### *F. OSHA's PBPK Analysis and Final Risk Estimates*

In developing an approach to PBPK modeling for MC, OSHA wished to use all of the available, appropriate and quantifiable biochemical and physiological data in its PBPK modeling and in assessing the uncertainty and variability in model parameters. The Agency determined that this approach would provide the best characterization of the variability and uncertainty in the data and the model. In addition, incorporation of as much of the available data as possible should give the most realistic PBPK model, and in turn, the most realistic risk estimate. Before development of OSHA's PBPK model, Clewell's approach (described above) was the most comprehensive pharmacokinetic approach submitted to the Agency. It addressed many of the issues of concern to the Agency, and OSHA believes that Clewell's approach was a reasonable template for using PBPK in risk assessment. However, since Clewell's work was done, PBPK modeling has continued to advance. Therefore OSHA modified Clewell's model to accommodate these advances and to allow incorporation of additional biochemical and physiological data that had been added to the rulemaking record. The following is a summary of OSHA's final (revised) PBPK analysis. A more detailed discussion can be found in the reports submitted to the Agency, reflecting OSHA's analysis in which the Agency was assisted by contractors [Ex. 128].

##### *1. Review of Clewell's PBPK Analysis*

a. Clewell's analytical approach. Clewell et al. [Ex. 96] employed Monte Carlo techniques to investigate imprecision in estimates of human health risk from occupational exposure to MC, as a function of imprecision in parameter values of the PBPK and dose-response models. (As described below, OSHA and its contractors believe that Clewell et al. did not correctly parse out uncertainty and variability, so their

analysis is described as accounting for "imprecision" rather than uncertainty or variability). In the Clewell et al. analysis, probability distributions were specified for each PBPK model parameter in an attempt to characterize imprecision. Computer-based techniques were used to obtain pseudo-random samples from these statistical distributions, generating multiple sets of model parameter values. These sets of parameter values were then used to obtain a corresponding distribution of PBPK model predictions of various measures of internal dose for a simulated animal bioassay (e.g., GST metabolism in lungs of mice exposed to 2000 ppm and 4000 ppm for 6 hrs/day, 5 days/wk). The mean of the mouse internal dose distribution was used as the dose input to obtain the MLE and UCL on the multistage model parameters, using the tumor incidence data from the NTP bioassay. The multistage model was run a second time using the upper 95th percentile of the mouse internal dose distribution as the dose input to obtain the MLE and UCL on the multistage model parameters. This yielded a total of four estimates of the parameters ( $q_0$ ,  $q_1$ , and  $q_2$ ) of the mouse dose-response function: 1) Mean of internal dose distribution/MLE of multistage model parameters; 2) Mean of internal dose distribution/UCL of multistage model parameters; 3) Upper 95th percentile of internal dose distribution/MLE of multistage model parameters; and 4) Upper 95th percentile of internal dose distribution/UCL of multistage model parameters.

Each set of dose-response parameters obtained from the analysis of the mouse data was then used to calculate human risk estimates. The upper 95th percentile of the human internal dose distribution was used to calculate the dose surrogate at 25 ppm, 8 hr/d exposure and then substituted into the MLE and UCL of the multistage parameters to obtain the MLE and UCL estimates of risk. Similarly the mean of the human internal dose distribution was used in conjunction with the MLE and UCL of the multistage model parameters. Therefore, four human risk estimates were generated, based on the distribution of human internal doses and the dose-response function derived from the multistage analysis of the NTP mouse bioassay. The four human risk estimates are: 1) upper 95th percentile of the human internal dose distribution/ MLE of the multistage model parameters; 2) mean of human internal dose distribution/ MLE of the multistage model parameters; 3) upper 95th percentile of the human internal dose

distribution/UCL of the multistage model parameters; and 4) mean of the human internal dose distribution/UCL of the multistage model parameters.

A major finding of that analysis was that the mean estimate of added cancer risk for occupational exposure at the proposed PEL of 25 ppm based on the PBPK-derived GST-lung dose surrogate (PBPK(mean) / potency(MLE) =  $0.39 \times 10^{-3}$ ) was 6-fold lower than the corresponding OSHA estimate (MLE =  $2.32 \times 10^{-3}$ ) based on administered dose scaled to body weight. The 95 percentile upper bound estimate of risk using the same PBPK distributions and the distribution of 95%UCLs on carcinogenic potency (PBPK(95%)/potency(95%) =  $1.56 \times 10^{-3}$ ), was nearly 2-fold less than OSHA's 95%UCL on risk ( $2.97 \times 10^{-3}$ ).

*b. Clewell's PBPK model.* The PBPK model used by Clewell et al. in performing their Monte Carlo analysis was slightly modified from the PBPK model developed by Andersen et al. and submitted to OSHA by HSIA [Ex. 328]. The primary modification was the addition of a separate compartment for the GI-tract. The general structure of this model has received considerable use by PBPK modelers. Nevertheless, there were several deficiencies in this model and in the subsequent statistical analysis that the Agency believed warranted major modification. These are described in the following section.

*c. Prior distributions for model parameters.* Truncated normals were used as the form for all probability distributions except for metabolic constants, which were described by truncated lognormals. All distributions were truncated to prevent sampling of nonsensical values (e.g., negative values). Truncation in some instances was 2 standard deviations (SDs) from mean values, in others more than 4 SDs.

A variety of sources of information were used as a basis for the probability distributions of the PBPK parameters in Clewell's model: literature summaries for most physiologic and anatomic parameters, direct laboratory measurement of partition coefficients based on vial equilibration studies, and statistical regression analyses of experimental data for fitted metabolic constants.

Clewell et al. stated that the focus of their analysis was on characterizing the effect of "uncertainty" in parameter values on uncertainty in PBPK model predictions, uncertainty being defined as the possible error in estimating the "true" value of a parameter for a representative "average" animal. To maintain consistency with a focus on investigating effects of parameter

uncertainty, a logical choice would have been to center their probability distributions using estimates of mean values for all model parameters and to use the standard error of the mean (SEM) to characterize dispersion. It is unclear whether this was done for blood flows, tissue volumes, inhalation rates or cardiac output, since Clewell et al. appear to have relied extensively on an unpublished review of scientific literature performed by S. Lindstedt for the ILSI Risk Science Institute Physiological Parameter Working Group.

Based on Clewell's comments accompanying his PBPK model, it appears that standard errors were not used to characterize variability among individual replicates of measured equilibrium partition coefficients; instead, standard deviations were used. Nor does it appear that Clewell et al. consistently made use of standard errors in characterizing imprecision in their fitted metabolic constants. Inspection of the joint confidence region for their fitted estimates of mouse VmaxC and Km (for the MFO pathway), shown in Figure 6 of Ex. 399, suggest coefficients of variation (%CVs) for VmaxC of about 2%. Similarly, for KfC, the %CV in the fitted MLE appears to be about 3%. These %CVs are considerably smaller than the assumed values of 20% and 30%, respectively, used by Clewell et al. in their Monte Carlo analysis. On the other hand, their %CV for Km does coincide with that indicated by the joint confidence regions. One should also note the high degree of correlation among the fitted values for VmaxC and Km.

In assessing variability in the ratio of *in vitro* MFO and GST metabolism in lung versus liver tissue (i.e., the A1 and A2 parameters), Clewell et al. used the *in vitro* MC metabolism data of Reitz et al. (1989). Yet it appears that the %CV for these data is 24% when one uses SDs among replicates for MFO metabolism in lung and liver of mice. This is substantially less than the 50% assumed by Clewell. One obtains a %CV of 9% when using SEMs.

It appears then, that some of the probability distributions used by Clewell et al. reflect variability beyond that readily identifiable as uncertainty in estimates of sample means. It may be that Clewell made a subjective inflation of variances. Though ad hoc, inflating variances would not be unreasonable given the sparse data on certain model parameters. Another possibility is that the distributions reflect variability due to both uncertainty and intersubject heterogeneity—another reason to inflate variances, or alternately, use SDs rather

than SEMs to describe the distributions of the parameters. If so, then it might be more appropriate to view the proportion of simulated estimates of risk that fall within a specified interval as the probability that the true risk for a randomly selected individual is in that interval. Yet strictly speaking this would require that the probability distributions reflect both the full range of uncertainty and heterogeneity in the population of interest, with the latter being unlikely based on inspection. If the analysis only considered imprecision due to uncertainty, as suggested in Clewell et al., then the resulting distribution should instead be viewed as describing the uncertainty in risk for a hypothetical "average" individual.

## 2. OSHA's Modifications to PBPK Analysis

*a. Basis for modifying approach of Clewell et al.* In addition to the likelihood that Clewell et al. used broader distributions than those necessary to model uncertainty in the PBPK analysis (as opposed to modeling some hybrid of uncertainty and variability), the analytical approach they used (1992 and 1993) also has two well-known methodological limitations. Their representation of imprecision in fitted parameters (e.g., VmaxC, Km, KfC) is problematic because they estimated the variability in these parameters by optimizing the model fit to *in vivo* data, while assuming nominal values for all other model parameters. However, the organ volumes, blood flows, and partition coefficients for the mice used in the gas uptake studies and the humans used in the open chamber studies are clearly not known with exact precision, and are not, therefore, accurately represented by nominal values. Consequently, the variances of the fitted parameters will be *underestimated* with this approach, since full acknowledgment of variability in other model parameters will have been ignored. Furthermore, it is quite likely that the joint parameter space for fitted PBPK model parameters will exhibit a considerable degree of correlation. Importantly, failure to account for such covariances when performing Monte Carlo sampling may *overstate* variance in some model predictions by assuming independence where it does not exist. The implications of these methodological limitations on predicted risk are unclear, since they would seem to exert countervailing effects on estimating uncertainty. Thus, OSHA decided that it was important to perform an analysis that addressed these limitations. The